

The Effect of Activating the Medial Olivocochlear Fibers on Cochlear Distortions in Humans

By

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Abstract

The function of the medial olivocochlear (MOC) fibers has been investigated extensively in animals, and far less in humans. A possible function of the MOC efferents is protection against loud sounds. The aim of this study is to investigate a potential tool for evaluating the MOC reflex clinically in humans. Cochlear microphonic (CM) and the associated distortions were measured while activating the MOC fibers for an extended period of time. CM was recorded in 16 normal hearing young adults using 500 Hz toneburst at 80 dB nHL. Recording of CM was conducted every three minutes for a time-block of 18-minutes. Four total 18-minute time-blocks were recorded, two without contralateral broadband noise (CBBN) [condition (1)] and two with 50 dB SPL CBBN [condition (2)]. The CM responses were subjected to fast-Fourier transform to obtain the amplitude of the primary frequency ($F_1=500\text{Hz}$), and the second ($2F_1=1000\text{Hz}$) and the third ($3F_1=1500\text{Hz}$) harmonics. A repeated-measures ANOVA was completed on the amplitude of F_1 , $2F_1$, and $3F_1$, and post-hoc analysis was utilized using LSD. There is approximately 21% increase in the F_1 amplitude as a result of presenting CBBN, which is significant ($p<0.01$). There is a significant change in $2F_1$ ($p<0.01$) and $3F_1$ ($p<0.01$) amplitudes as a result of presenting the CBBN. The current study shows that the activation of the MOC fibers results in enhancement of the CM response. Furthermore, the results show that activation of the MOC fibers causes modulation of $2F_1$ and $3F_1$ of the CM response. The resulting changes of the CM distortions are in agreement with the proposed model of adjusting the operating point of the cochlear amplifier as a result of activating the MOC fibers. These results support the use of CM measurement as an objective measure for evaluating the MOC reflex clinically.

KEYWORDS: Outer hair cells, cochlear amplifier, cochlear microphonic, medial Olivocochlear fibers, auditory efferent system, cochlear distortions.

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Chapter I: Introduction

The olivocochlear bundle (OCB) is a neural pathway that descends from the brainstem to the cochlea as part of the auditory pathway. The descending pathway is not understood as well as the ascending pathway. Researchers have been interested in studying the function of the OCB to better understand its role in the auditory system (Desmedt, 1962; Tavartkiladze, Frolenkov, & Artamasov, 1996). The function of the OCB has been investigated extensively in animals, and far less in humans. As a result, there are two proposed functions associated with the OCB: improving speech understanding in noisy environments and protecting against loud sounds (Guinan, 2006). Furthermore, Maison and Liberman (2000) showed that guinea pigs with strong OCB activity are less susceptible to cochlear damage after being exposed to loud sounds. These findings warrant the development of a clinical tool to evaluate the OCB in humans.

The OCB originates in the superior olivary complex and innervates the cochlea through two pathways: the medial olivocochlear (MOC) neurons that innervates the cochlear outer hair cells (OHCs), and the lateral olivocochlear (LOC) neurons that innervates the Type I afferent fibers that synapse with the cochlear inner hair cells (Guinan, 2006). When the OCB is activated, it suppresses the cochlear responses (Desmedt, 1962; Puria, Guinan, & Liberman, 1996). As described in the literature, there have been two different methods used in activating the OCB: applying electrical current to the floor of the 4th ventricle in the brainstem, or presenting an acoustical signal, noise or pure tone, to the ipsilateral ear, the contralateral ear, or both. Several methods are used to measure the effect of activating the OCB on cochlear function, including the use of: otoacoustic emissions (OAEs), the auditory nerve compound action potential (CAP), and the cochlear microphonic (CM).

To evaluate the OCB in humans—the MOC reflex in particular—OAEs are considered the gold standard because they are quick and easy to measure using noninvasive recording approaches. Limited studies in humans reveal that activation of the MOC fibers results in amplitude suppression of OAEs (Berlin, Hood, Hurley, Wen, & Kemp, 1995; Tavartkiladze et al., 1996). However, these effects are very small and somewhat controversial. For example, Berlin et al. (1995) reported that ipsilateral activation of the MOC neurons results in about 0.5 to 1 dB of suppression of the transient evoked otoacoustic emission (TEOAE), while contralateral activation of the MOC neurons results in about 0.5 dB of suppression of the TEOAE. Abdala, Mishra, and Williams (2009), on the other hand, reported about 2 dB suppression to the distortion product OAEs (DPOAE) when the MOC fibers were contralaterally activated. In yet another study, Abel, Wittekindt, and Kössl (2009) reported that activating the MOC efferents contralaterally does not result in suppression of DPOAE (i.e. $2F_1-F_2$), which is the test commonly used in clinic. Another limitation associated with using OAEs to assess MOC fibers function is the interfering effects of background noise, especially at lower frequencies (Wittekindt, Gaese, & Kössl, 2009). Wittekindt et al. (2009) attempted to study the MOC reflex in humans using the DPOAE (i.e. F_2-F_1), and reported that the lowest frequency that could be tested was 833 Hz due to the background noise. Additionally, OAEs cannot be recorded from subjects with minimal or subclinical damage to the cochlea. This characteristic limits the use for OAEs in evaluating the MOC reflex for this population.

Lastly, when the OHC is at rest, the mechanoelectrical transduction (MET) channels on the stereocilia have high probability of being closed. However, in the presence of sound, the stereocilia start oscillating back and forth around the operating point, which corresponds with the zero pressure input on the OHC transducer curve. These oscillations result in changes to the

open probability of the MET channels on the hair cells stereocilia, and in result, controlling the amount of K^+ current entering the hair cell to depolarize it. During these sinusoidal oscillations, the stereocilia pass through the operating point when the sound wave has a zero pressure point. Shifting the operating point from to a new position will affect the open probability of the MET channels as well as the cochlear distortions. The change in the OHCs operating point position is not easily recorded through measuring the clinically recorded DPOAE (i.e. $2F_1-F_2$), which is another limitation to using OAEs to evaluate the MOC fibers function. A study by Brown, Hartsock, Gill, Fitzgerald, and Salt (2009) revealed that manipulation of the organ of Corti operating point showed very small effects on the amplitude of the $2F_1-F_2$. The significance of this limitation relates to the possible function of the MOC fibers investigated in our study.

Given the above mentioned limitations associated with using OAEs in studying the MOC reflex, the CM has drawn attention as an alternate cochlear response that can be used for this purpose. It has been long known that activation of the MOC fibers causes the CM response to increase in amplitude (Desmedt, 1962). In addition, the MOC efferents appear to have larger effect on evoked potentials compared to other responses, such as OAEs (Puria et al., 1996; Zeng, McFadden, Henderson, Ding, & Burkard, 2000). Furthermore, changes to the even order cochlear distortions as a result of changing the operating point showed a larger modulation when the CM (i.e. $2F_1$) was used (Brown et al., 2009).

Another advantage to the use of the CM was shown by Jamos et al. (2012). These researchers investigated the activation of the MOC neurons on the CM, and showed that the effect is greater at lower frequencies compared to higher frequencies. Therefore, recording the CM can be advantageous because it would give researchers a window to evaluate the MOC reflex at lower frequencies, which is an area that has been lacking in other studies. Lastly, the

CM response is more resilient than OAEs in cases of minimal degrees of cochlear hearing loss (Santarrelli, Scimemi, Dal Monte, & Arslan, 2006).

The importance of measuring the MOC reflex arises from the assumption that it would allow clinicians to identify individuals not performing well in noisy environments, or if they would be at a higher risk for noise induced hearing loss. Therefore, identifying a reliable tool to evaluate the MOC reflex in humans can provide valuable information for clinicians. The aim of this study is to investigate a potential tool for evaluating the MOC reflex clinically in humans; specifically, through evaluating the effect of activating the MOC fibers on cochlear distortion products measured via the CM response. This tool can provide more information about the physiological changes at the cochlear level as a result of stimulating the MOC neurons. The proposed procedure included recording of the CM response to 500 Hz stimulus with and without presenting the MOC activator, broadband noise, to the contralateral ear. Presentation of the MOC activator was for an extended period of time, and the CM response was recorded at different points in time to evaluate the time course of the MOC reflex. This study focused on the cochlear distortions recorded by the CM response. The changes to even ($2F_1$) and odd ($3F_1$) order distortions as a result of activating the MOC efferents were investigated. The null hypothesis for the proposed study states that presentation of the MOC activator over an extended period of time will not have any additional impact on odd nor even order distortions of the CM response. The alternative hypothesis states that the amount of modulation of odd and even order distortions of CM response will increase with prolonged stimulation of the MOC fibers.

Chapter II: Methods

The study was conducted on 16 young adult females with mean age 23 years (range 20 – 30 years). All subjects met the inclusion criteria, which includes: (1) intact tympanic membrane and clear ear canals; (2) normal middle ear function; (3) contralateral (Re: probe ear) acoustic reflex threshold above 65 dB SPL for broadband noise, acoustic reflex threshold is defined as the lowest level to evoke a response; (4) normal hearing function (< 25 dB HL) at all frequencies between 250 and 8000 Hz; (5) present CM response with no stimulus artifact. Several reports indicate that the right ear shows larger amount of suppression compared to the left ear (Gkoritsa et al., 2007; Khalfa & Collet, 1996; Khalfa, Morlet, Micheyl, Morgon, & Collet, 1997), therefore, the current study focused on collecting data from the right ear of all subjects.

All experimental procedures were conducted in a sound treated booth at the Auditory Research Laboratory at Missouri State University. The middle ear function and the acoustic reflexes were evaluated using Grason-Stadler (GSI) TymStar middle ear analyzer. Hearing thresholds were measured using Grason-Stadler Instrument 61 audiometer and insert earphones. The intelligent hearing systems (IHS) Smart-Evoked Potential (SmartEP) was used to record the CM response. The recording of the CM response was completed using self-manufactured tympanic membrane (TM) electrodes and disposable surface electrodes. The self-manufactured TM electrodes were constructed as described by Ferraro and Durrant (2006). All equipment was checked and calibrated before data collection began.

The CM recording was completed using a 500 Hz toneburst (TB) stimulus; therefore, the even order distortion frequency ($2F_1$) was 1000 Hz, and the odd order distortion frequency ($3F_1$) was 1500 Hz. The 500 Hz TB stimulus was presented at 80 dB nHL. The MOC fibers were activated using a CBBN stimulus presented at 50 dB SPL. Before starting data collection from

each subject, a control run, as described below, was completed to confirm that the CM response is a true cochlear response. The recording of CM was completed in two conditions: Condition (1)—recording CM every three minutes for a total of 18-minute long time-block allowing for seven recordings without presenting CBBN as shown in Figure 1-top; Condition (2)—recording CM every three minutes for a total of 18-minute long time-block allowing for five recordings with presenting CBBN (0, 3, 6, 9, and 12 minutes), and two recording after the CBBN was turned off (15 and 18 minutes), as shown in Figure 1-bottom. In condition (2), the CBBN was presented continuously for the first 12 minutes. Each condition was repeated twice to confirm repeatability. Analysis was conducted on the average of the two time-blocks of each condition, with or without CBBN. An additional 5-minute break was utilized between each two time-blocks. This design allowed us to measure changes in even and odd order distortions over time.

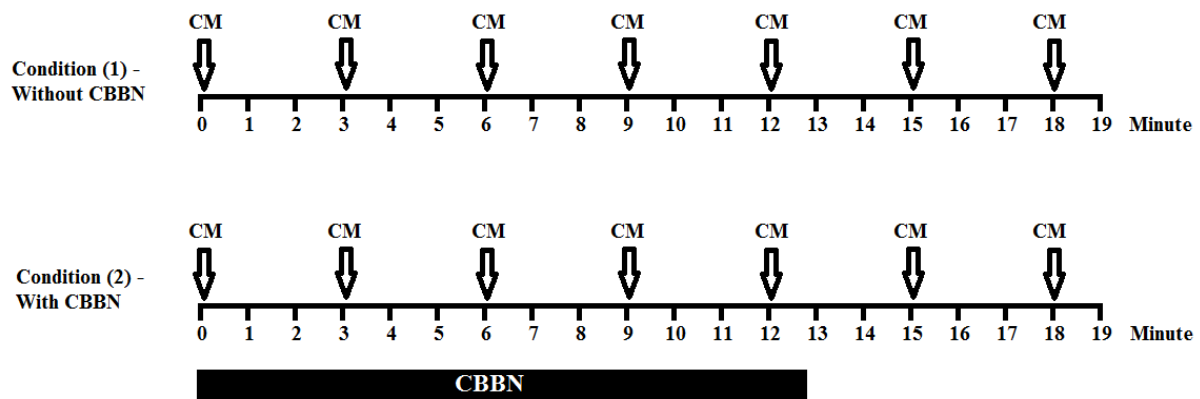


Figure 1. Experimental paradigm for CM recording. The top part of the graph represents condition (1), which indicates seven CM recording completed every three minutes without presenting CBBN. The bottom part of the graph represents condition (2), which indicates five CM recording completed every three minutes with presenting CBBN, and two CM recordings (separated by three minutes) after turning off the CBBN. The thick line represents the continuous presentation of CBBN, which continued for 12 minutes.

It should be noted that with presenting 500 Hz TB at a loud level (i.e. 80 dB nHL), there is a high chance for creating harmonic distortions in the stimulus itself, which might be a threat

to the validity of our recordings. Therefore, a small experiment was completed before data collection to address this issue. The stimulus levels of the fundamental frequency (500 Hz) and the second and third harmonics (1000 and 1500 Hz) were measured in the ear canal using Etymotic Research 7C probe microphone (sensitivity of 50 mV/Pa), which was connected to Tektronix mixed domain oscilloscope (MDO3022). After identifying the harmonic levels in the ear canal, CM recordings were conducted for 1000 and 1500 Hz at those measured levels. The hypothesis of this experiment was that if a CM response was evoked using the levels measured in the ear canal for both frequencies, then the distortions present in the response to a 500 Hz TB will be in question. On the other hand, if there is no CM response present at the ear canal harmonics level, then the distortions present in the CM response to 500 Hz are a result of the cochlear nonlinearity would be supported. The aforementioned procedure was described by Peter Dallos (1973). The ear canal measurements and CM recording were completed in a 29-year-old subject. When the 500 Hz TB stimulus was presented at 80 dB nHL ($1.81 \text{ V} = 125 \text{ dB pSPL}$), a second harmonic distortion (i.e. 1000 Hz) of 30 dB nHL ($-51 \text{ dB re. } F_1$) was recorded in the ear canal. Then, CM response, shown in Figure 2, was recorded using a 1000 Hz TB stimulus at 30 dB nHL, and revealed no response. This finding supports the hypothesis that the distortions measured using 500 Hz are resulting from the cochlear nonlinearity.

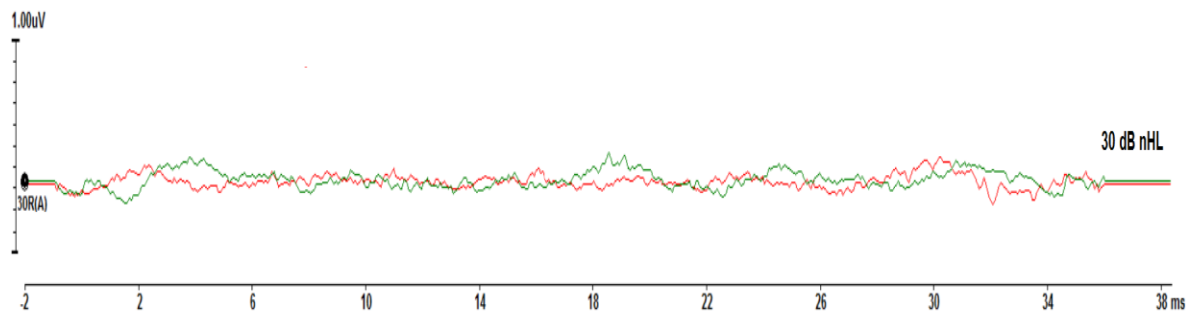


Figure 2. CM response to a 1000 Hz TB at 30 dB nHL. The recording indicates that 30 dB nHL is not loud enough to evoke a CM response.

The CM responses were recorded using a horizontal montage with the non-inverting (+) electrode at the ipsilateral tympanic membrane, the inverting electrode at the contralateral mastoid, and the ground at mid forehead. The recording was conducted on the right ear of all subjects using an 80 dB nHL 500 Hz TB stimulus with 4 msec rise/fall time and 20 msec plateau (2-10-2 cycles). Each trace was recorded as an average of 1024 rarefaction stimuli presented at a rate of 27.1 Hz. The responses were recorded with a 100–5000 Hz bandpass filter and were amplified 100,000 times. The CBBN was presented at 50 dB SPL. Before presenting the CBBN, the CM response was recorded and confirmed as a true CM response; therefore, a control run was completed while the insert earphone tube was pinched to make sure that there was no response. The presence of sinusoidal response similar to the CM response while the tube was pinched would have indicated that the recording was a stimulus artifact. Additional measures were utilized to reduce the chance for recording stimulus artifacts, including: increasing the length of the insert earphone tube to keep it away from the recording electrodes and wrapping the insert earphones with tinfoil to enhance insulation.

Each CBBN time-block started with a simultaneous presentation of broadband noise in the contralateral ear and 500 Hz TB in the ipsilateral ear ($t = 0$); then, a trace was recorded every three minutes ($t = 3$, $t = 6$, $t = 9$, $t = 12$) while the CBBN was continuously presented, and two more traces were recorded ($t = 15$, $t = 18$) after the CBBN was turned off. Subjects were encouraged not to fall asleep during the recording session to eliminate any effects of the arousal state (Froehlich, Collet, Valtax, & Morgan, 1993). The four time-blocks [two for condition (1) and two for condition (2)] were randomized using the Latin Square design. The proposed procedure was approved by the institutional review board (IRB) on 10/24/2016; confirmation number: IRB-FY2017-319.

Statistical Methods

Power analysis to identify the required sample size was conducted using G*Power 3 software. The analysis was based on an effect size partial $\eta^2 = 0.07 - 0.09$ that was found in the study conducted by Jamos et al. (2012) after analyzing the power spectrum results. Based on this value, to obtain a power of 0.96, a sample size between 11 and 14 subjects would be needed (based on the effect size used). A total of 16 subjects were recruited.

This study is a within-subject design with two independent variables: CBBN presence and time of recording. The CM response power spectrum was analyzed using the response fast Fourier transformer (FFT) function of the IHS SmartEP system used in the recording. The analysis was conducted over a frequency range of 1 to 2000 Hz. Descriptive statistics of the F_1 , $2F_1$, and $3F_1$ amplitudes, and phase shift are provided. The analysis was completed for four dependent variables (F_1 , $2F_1$, and $3F_1$ amplitudes, and phase shift). The statistical analysis was completed using repeated-measures analysis of variance (ANOVA) for each dependent variable. Post-hoc comparisons were conducted to analyze the differences between conditions using LSD.

Chapter III: Results

The recording of CM was completed from 16 normal-hearing young adult females ranging from 20 to 30 years old (mean = 23 years). As shown in Figure 3(a), all subjects had hearing within normal limits (≤ 10 dB HL) at all frequencies from 250 to 8000 Hz. Also, Figure 3(b) shows that all subjects had present and normal acoustic stapedial thresholds (ART) for an ipsilateral 500 Hz toneburst (mean = 91 dB HL), as well as CBBN (mean = 82 dB HL). The ARTs were present at levels higher than the stimuli used in the experiment for all subjects. Having the ART higher than the stimuli presented in the experiment helped rule out the involvement of the stapedial muscle in the changes observed in the study.

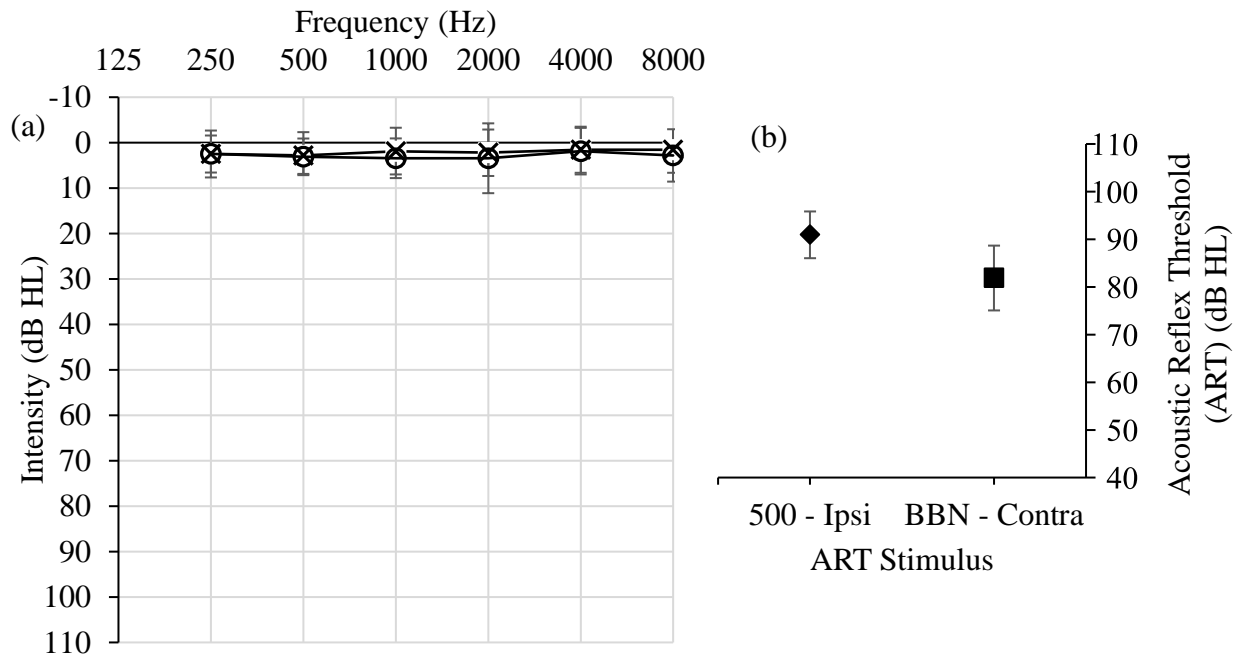


Figure 3. Hearing threshold and ART for subjects in the study. (a) Represents grand average of hearing threshold at each frequency between 250 and 8000 Hz [right ear (o) and left ear (x)]. (b) Represents ART for CBBN and ipsilateral 500 Hz in reference to the right ear (i.e. right ear is the probe ear). Error bars represent standard deviation (± 1 SD).

For each subject, a baseline CM recording was performed and repeated before any experimental recordings were completed. Also, a control run was completed through pinching the tube to confirm the presence of an actual CM response. The experimental recording was then completed by recording the CM response in the different conditions: condition (1)-without CBBN, and condition (2)-with CBBN. Two time-blocks for condition (1) were completed without stimulating the CBBN, and two blocks were completed while presenting CBBN for the first 12-minutes of each block—condition (2). Recordings were completed at 0, 3, 6, 9, 12, 15, and 18 minute marks. Figures 4(a) and 4(b) show the recordings without and with CBBN for one subject, as well as the baseline and the control runs. After recording of the CM response, analyses of the FFT responses were conducted at the primary frequency and its second and third harmonics.

Overall Group CM Results

Primary frequency (i.e. $F_1 = 500$ Hz). Amplitude of F_1 was identified for each recording. For the recordings without CBBN, the amplitude of the associated recordings from each time-block were averaged (i.e. 0-minute recording from first time-block without CBBN and 0-minute recording from second time-block without CBBN); the same was completed for the recordings with CBBN. Repeated measures ANOVA was conducted to compare each recording without CBBN to baseline. Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2(27) = 139.766, p < 0.001$. Therefore, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.207$). As illustrated in Figure 5 (dashed lines), the results show that F_1 amplitude was not significantly different at any recording compared to baseline [$F(1.448, 21.716) = 0.742, p = 0.447, \eta^2 = 0.047$].

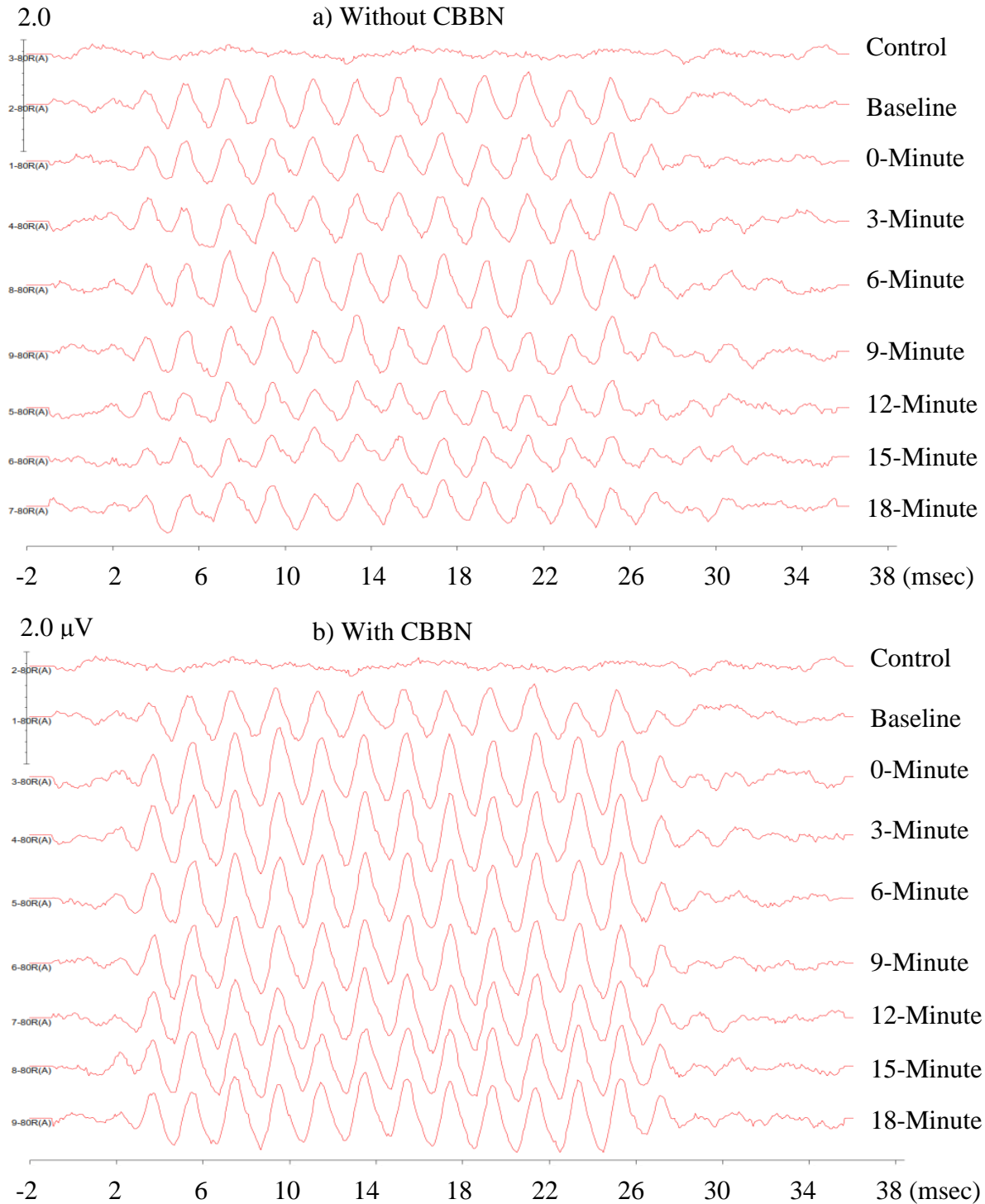


Figure 4. Recordings from subject # 12. (a) Shows CM recordings without CBBN, as well as the control and baseline recordings. (b) Shows CM recordings with CBBN, as well as the control and baseline recordings from the same subject. The recording mark within each time-block is on the right end of each trace.

As for the recording with CBBN, repeated measures ANOVA was conducted to compare F_1 amplitude for each recording with CBBN to baseline. Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2(27) = 88.39$, $p < 0.001$. Thus, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.34$). The results shown in Figure 5 (solid line) demonstrate that F_1 amplitude was significantly different compared to baseline [$F(2.381, 35.722) = 5.543$, $p < 0.01$, $\eta^2 = 0.27$]. Post-hoc analysis of CM recordings with CBBN revealed that F_1 amplitude was significantly different at 0-, 3-, 6-, 9-, and 12-minute marks, in comparison to baseline, but not at 15- and 18-minute marks, illustrated in table 1. These significant changes occurred during the presentation of CBBN (thick straight line-Figure 5). Also, after turning off CBBN, F_1 amplitude did not return completely to baseline.

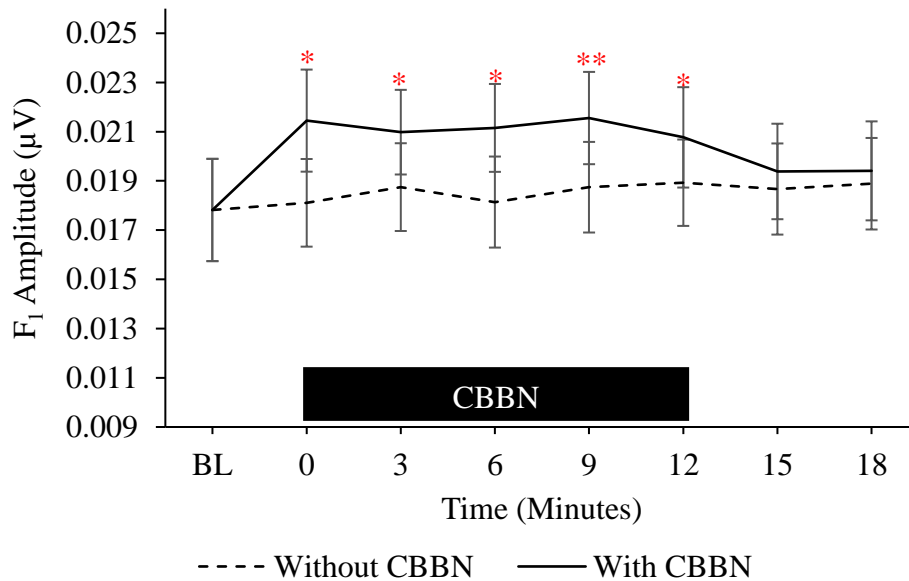


Figure 5. Mean of F_1 amplitude baseline (BL) recording, as well as the seven recording marks without (dashed line) and with CBBN (solid line). Asterisks represent the significantly different recordings in comparison to BL. The graph shows that F_1 amplitude increased with the presence of CBBN, and dropped close to BL after CBBN was turned off (i.e. minutes 15 and 18), but not all the way back to BL. F_1 amplitude did not significantly change from BL in the absence of CBBN. The thick line at the bottom of the graph represents the duration of presenting CBBN, in the condition (2). (* $p < 0.05$, ** $p < 0.01$). Error bars represent standard error (± 1 SE).

Table 1. Post-hoc analysis of F_1 amplitude without and with the presence of CBBN. Comparison of F_1 amplitude at each recording mark to baseline (mean = 0.0178 μV).

Without CBBN			With CBBN		
Recording Mark	Mean (μV)	<i>p-value</i>	Recording Mark	Mean (μV)	<i>p-value</i>
0-Minute	0.0181	.81	0-Minute	0.0214	.017*
3-Minute	0.0187	.40	3-Minute	0.0210	.016*
6-Minute	0.0181	.79	6-Minute	0.0212	.014*
9-Minute	0.0187	.44	9-Minute	0.0216	.008**
12-Minute	0.0189	.39	12-Minute	0.0208	.044*
15-Minute	0.0187	.53	15-Minute	0.0194	.24
18-Minute	0.0189	.46	18-Minute	0.0194	.18

* $p < 0.05$, ** $p < 0.01$

Second harmonic (i.e. $2F_1 = 1000 \text{ Hz}$). Next, the amplitude of $2F_1$ was identified for each recording. For the recordings without CBBN, the amplitude of the associated recordings from each time-block were averaged; the same was completed for the recordings with CBBN. Repeated measures ANOVA was conducted to compare each recording without CBBN to baseline. Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2(27) = 98.749$, $p < 0.001$. Therefore, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.247$). As shown in Figure 6 (dashed line), the results revealed that $2F_1$ amplitude was significantly different compared to baseline [$F(1.73, 25.956) = 4.49$, $p < 0.05$, $\eta^2 = 0.23$]. Repeated measures ANOVA was also used to compare $2F_1$ amplitude of each recording with CBBN to baseline. Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2(27) = 59.531$, $p < 0.01$. Therefore, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.397$). As shown in Figure 6 (solid line), the results revealed that $2F_1$ amplitude was significantly different compared to baseline [$F(2.782, 41.737) = 5.471$, $p < 0.01$, $\eta^2 = 0.267$]. Post-hoc analysis of recordings without and with CBBN

revealed that 2F₁ amplitude was significantly different at all recordings in comparison to baseline, illustrated in table 2.

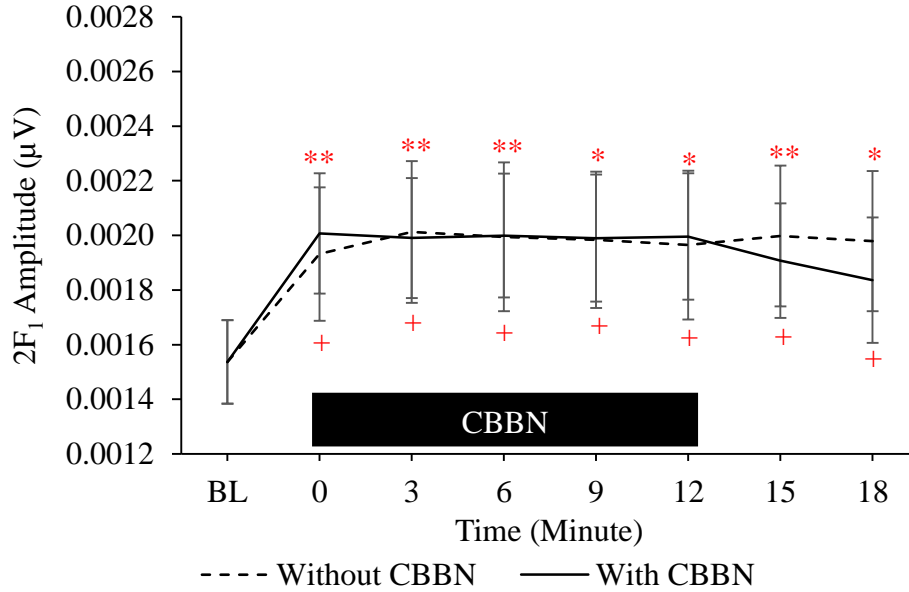


Figure 6. Mean of 2F₁ amplitude baseline (BL) recording, as well as the seven recording marks without (dashed line) and with CBBN (solid line). Pluses represent the significantly different recordings of the without CBBN in comparison to BL, and asterisks represent the significantly different recordings of the with CBBN in comparison to BL. The graph shows that 2F₁ amplitude increased for all recordings without and with CBBN. The thick line at the bottom of the graph represents the duration of presenting CBBN, in condition (2). (* [or +] $p < 0.05$, ** $p < 0.01$). Error bars represent standard error (± 1 SE).

Table 2. Post-hoc analysis of 2F₁ amplitude without and with the presence of CBBN. Comparison of 2F₁ amplitude at each recording mark to baseline (mean = 0.00154 μV).

Without CBBN			With CBBN		
Recording Mark	Mean (μV)	<i>p</i> -value	Recording Mark	Mean (μV)	<i>p</i> -value
0-Minute	0.00193	.025 ⁺	0-Minute	0.00201	.004 ^{**}
3-Minute	0.00201	.022 ⁺	3-Minute	0.00199	.004 ^{**}
6-Minute	0.00199	.037 ⁺	6-Minute	0.002	.006 ^{**}
9-Minute	0.00198	.028 ⁺	9-Minute	0.00199	.012 [*]
12-Minute	0.00196	.037 ⁺	12-Minute	0.002	.009 ^{**}
15-Minute	0.0020	.021 ⁺	15-Minute	0.00191	.002 ^{**}
18-Minute	0.00198	.037 ⁺	18-Minute	0.00184	.045 [*]

*[or +] $p < 0.05$, ** $p < 0.01$

Third harmonic (i.e. $3F_1 = 1500$ Hz). Next, the amplitude of $3F_1$ was identified for each recording. For the recordings without CBBN, the amplitude of the associated recordings from each time-block were averaged; the same was completed for the recordings with CBBN. Repeated measures ANOVA was conducted to compare each recording without CBBN to baseline. Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2(27) = 48.57, p < 0.01$. So, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.617$). Figure 7 (dashed line) shows that $3F_1$ amplitude was not significantly different compared to baseline [$F(4.317, 64.754) = 2.168, p = 0.078, \eta^2 = 0.126$]. Repeated measures ANOVA was used to compare $3F_1$ amplitude for each recording with CBBN to baseline. Mauchly's test indicated that the assumption of sphericity had been satisfied, $\chi^2(27) = 26.619, p = 0.51$. As illustrated in Figure 7 (solid line), the results revealed that $3F_1$ amplitude was significantly different compared to baseline [$F(7, 105) = 6.704, p < 0.01, \eta^2 = 0.309$]. Post-hoc analysis of recordings with CBBN revealed that $3F_1$ amplitude was significantly different at 0-, 3-, 6-, 9-, -12, and 15-Minute marks, in comparison to baseline, but not at 18-Minute marks, illustrated in table 3.

Table 3. Post-hoc analysis of $3F_1$ amplitude without and with the presence of CBBN. Comparison of $3F_1$ amplitude at each recording mark to baseline (mean = $0.00039 \mu V$).

Without CBBN			With CBBN		
Recording Mark	Mean (μV)	<i>p-value</i>	Recording Mark	Mean (μV)	<i>p-value</i>
0-Minute	0.00045	.07	0-Minute	0.00056	.0003**
3-Minute	0.0005	.007 [§]	3-Minute	0.00056	.00009**
6-Minute	0.00047	.075	6-Minute	0.00053	.0008**
9-Minute	0.00047	.09	9-Minute	0.00049	.019*
12-Minute	0.00047	.103	12-Minute	0.00052	.006**
15-Minute	0.00048	.008 [§]	15-Minute	0.00046	.033*
18-Minute	0.00044	.17	18-Minute	0.00044	.08

* $p < 0.05$, ** $p < 0.01$

[§]Those comparisons were discarded since the main effect was not significant.

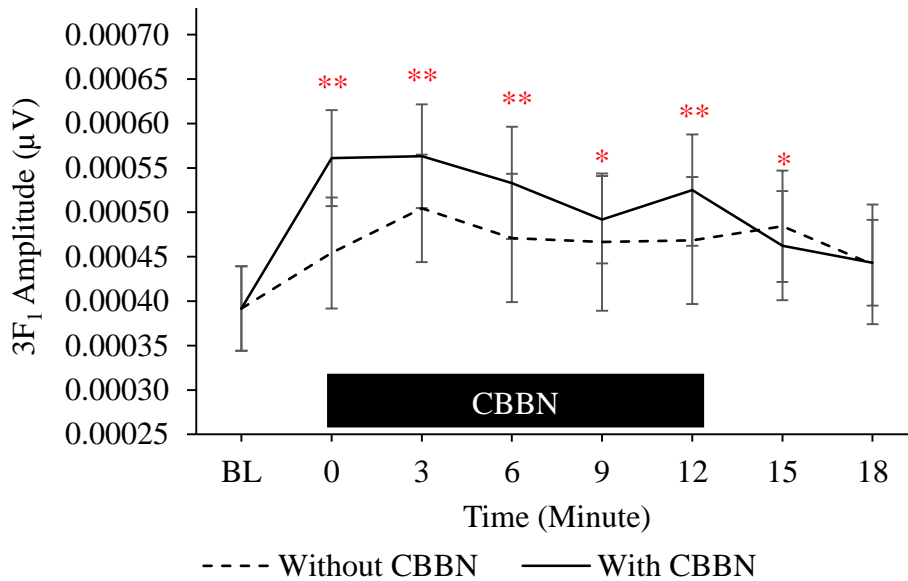


Figure 7. Mean of $3F_1$ amplitude baseline (BL) recording, as well as the seven recording marks without (dashed line) and with CBBN (solid line). Asterisks represent the significantly different measurements in comparison to BL. The graph shows that $3F_1$ amplitude increased with the presence of CBBN, and returned to baseline over time, and that $3F_1$ amplitude did not significantly change from BL in the absence of CBBN. The thick line at the bottom of the graph represents the duration of presenting CBBN, in condition (2). (* $p < 0.05$, ** $p < 0.01$). Error bars represent standard error (± 1 SE).

CM response phase shift. Interestingly, during the recording of CM responses, an observation was made on the CM response phase. Namely, that it appears to change over time. This observation, which is shown in Figure 8, led to additional analysis. A total of 12 out of 16 (75%) subjects had a phase shift, either leading or lagging in reference to baseline, by 10 degrees or more. The phase shift was calculated mathematically for each CM recording as follows: first, as illustrated in Figure 8, three peaks were identified starting at 10 msec. Second, the latency of each peak was subtracted from the corresponding peak latency of the baseline response; the resulting difference was then averaged for the three peaks to reduce variability of peak labeling; lastly, calculated the amount of phase shift through applying formula (1). Note that the baseline phase is considered 0 and the period of 500 Hz frequency is 2 msec.

$$\text{Phase angle} = 360 * \left(\frac{\text{Time difference}}{\text{Period}} \right) \dots\dots\dots \text{Formula (1)}$$

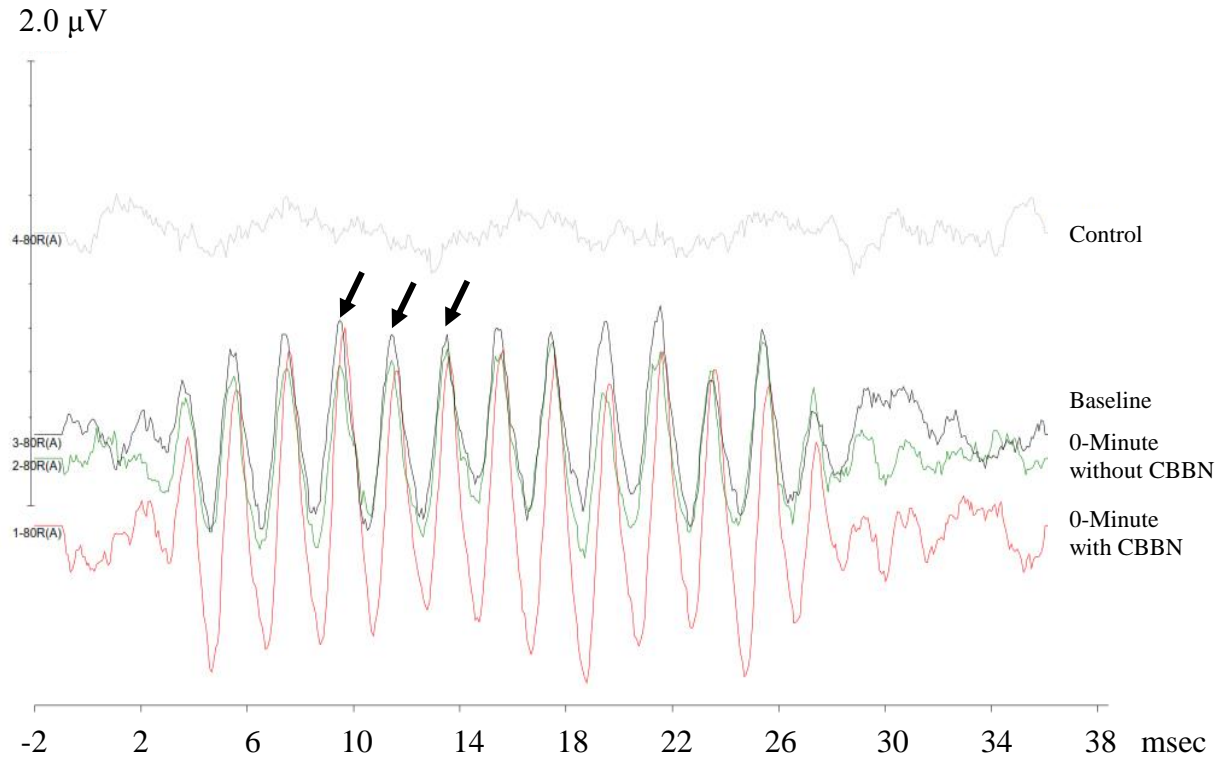


Figure 8. Shows the phase changes observed in the CM response of subject # 12. (Black) BL recording. (Green) 0-Minute mark recording without CBBN. (Red) 0-Minute mark recording with CBBN, which demonstrated a lagging phase shift in reference to the baseline. (Gray) recording represents the control run while the insert tube was pinched. Arrows represent the peaks used to calculate the phase shift.

For the purposes of analysis, the absolute value of the phase shift was used. Repeated measures ANOVA was used to compare the phase shift for each CM recording without CBBN to the baseline of 0 degrees (i.e. no shift). Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2(27) = 145.232$, $p < 0.01$. Consequently, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.229$). The results, illustrated in Figure 9

(dashed line), show that the phase shift was significantly different in comparison to baseline [$F(1.606, 24.084) = 9.093, p < 0.01, \eta^2 = 0.377$]. Repeated measures ANOVA was, then, used to compare the phase shift for each CM recording with CBBN to the baseline of 0 degrees.

Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2(27) = 136.057, p < 0.01$. Consequently, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.23$). The results, which are depicted in Figure 9 (solid line), show that the phase shift was significantly different compared to baseline [$F(1.607, 24.103) = 15.504, p < 0.01, \eta^2 = 0.508$]. Post-hoc analysis of recordings without and with CBBN revealed that phase shift was significantly different at all recordings in comparison to baseline, illustrated in table 4.

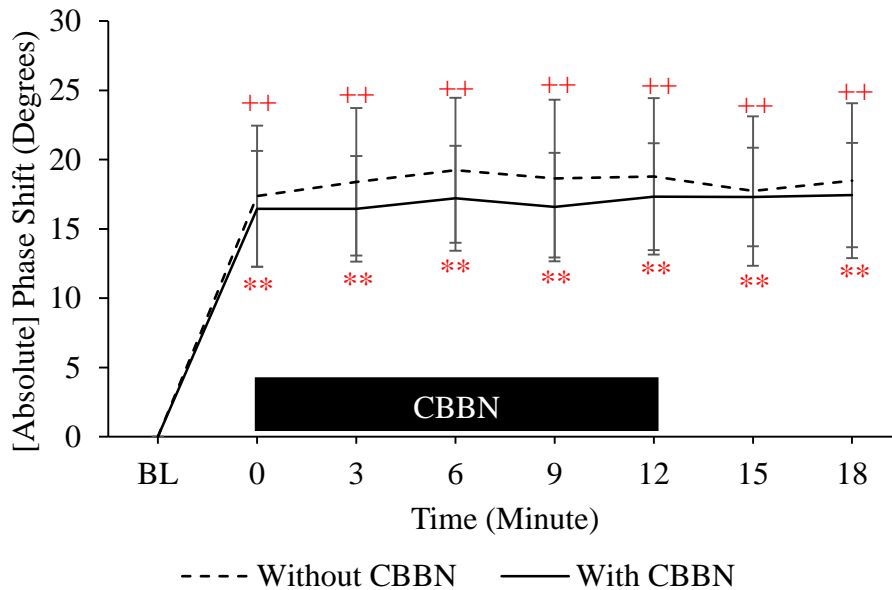


Figure 9. Mean of (absolute) phase shift of the CM response of the seven recording without (dashed line) and with CBBN (solid line). Pluses represent the significantly different measurements without CBBN, and asterisks represent the significantly different measurements with CBBN in comparison to BL. The graph shows that there was a phase shift observed in all recordings. The thick line at the bottom of the graph represents the duration of presenting CBBN, in condition (2). (** [or ++] $p < 0.01$). Error bars represent standard error (± 1 SE).

Table 4. Post-hoc analysis of phase shift without and with the presence of CBBN. Comparison of phase shift at each recording mark to arbitrary baseline of 0 degrees.

Without CBBN			With CBBN		
Recording Mark	Mean (deg)	<i>p-value</i>	Recording Mark	Mean (deg)	<i>p-value</i>
0-Minute	17.36	.004 ⁺⁺	0-Minute	16.44	.001 ^{**}
3-Minute	18.39	.004 ⁺⁺	3-Minute	16.44	.0006 ^{**}
6-Minute	19.24	.002 ⁺⁺	6-Minute	17.21	.0004 ^{**}
9-Minute	18.64	.005 ⁺⁺	9-Minute	16.58	.0007 ^{**}
12-Minute	18.79	.005 ⁺⁺	12-Minute	17.33	.0004 ^{**}
15-Minute	17.74	.005 ⁺⁺	15-Minute	17.31	.0002 ^{**}
18-Minute	18.49	.005 ⁺⁺	18-Minute	17.44	.0003 ^{**}

^{**} [or ⁺⁺] $p < 0.01$

Sub-group – Subjects Receiving Condition (1) First

During data collection, the four 18-minute time-blocks of recording (two time-blocks of condition (1)-without CBBN, and two time-blocks of condition (2)-with CBBN) were randomized. A total of 10 subjects had condition (1) presented first, while six subjects had condition (2) presented first. At the time of the design, and after reviewing the literature, this idea seemed like a good way to reduce bias. However, as shown in Figure 10, five out of 10 subjects who received condition (2) first appeared to show a carry-over effect. This carry-over effect appeared as an increase in CM amplitude that did not return to the baseline during the five minutes rest period given to subjects between the different time-blocks. Therefore, a similar analysis to the whole group data was conducted on a sub-group of subjects; specifically, the six subjects who received condition (1)—without CBBN—first. (Note. See appendix A for the sub-group subjects receiving condition (2) first)

Primary frequency for sub-group. Amplitude of F_1 was identified for each recording, similar to what was described above. Repeated measures ANOVA was conducted to compare each recording without CBBN to baseline. Mauchly's test significance could not be determined

due to the small sample size, therefore, and to be conservative, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.273$). The results illustrated in Figure 11 (dashed line) show that F_1 amplitude was not significantly different at any recording compared to baseline [$F(1.909, 9.544) = 2.021, p = 0.186, \eta^2 = 0.288$].

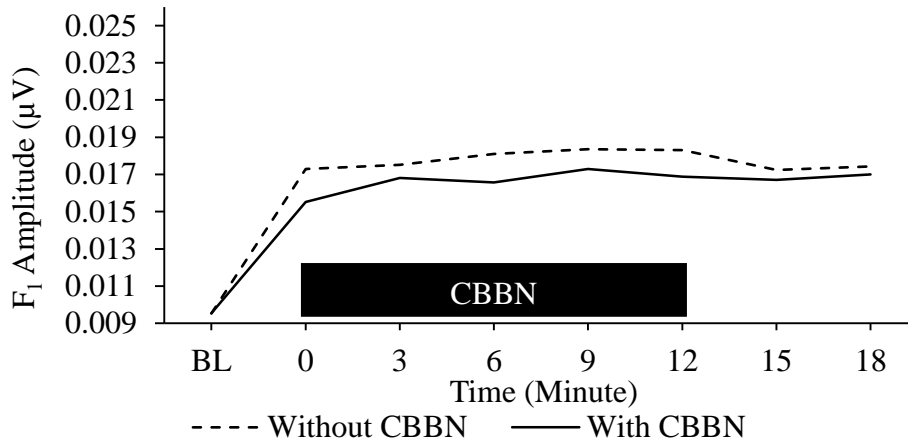


Figure 10. Subject #8 – F_1 amplitude of the seven recordings with condition (2) measured first (solid line), and condition (1) second (dashed line). The results showing the carry-over effect with amplitude not returning to the baseline after turning off the CBBN, and giving the subject 5-minute break before starting to measure CM without CBBN.

Repeated measures ANOVA was conducted to compare recordings with CBBN to baseline. Mauchly's test significance could not be measured due to the small sample size, therefore, and to be conservative, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.245$). As shown in Figure 11 (solid line), the results show that F_1 amplitude was significantly different compared to baseline [$F(1.713, 8.566) = 5.759, p < 0.05, \eta^2 = 0.535$]. Post-hoc analysis revealed that F_1 amplitude was significantly different at 0-, 3-, 6-, and 9-minute marks, in comparison to baseline, but not at 12-, 15-, and 18-minute marks, illustrated in table 5. These significant changes occurred during the presentation of CBBN. Also, after turning off CBBN, F_1 amplitude did not return completely to baseline.

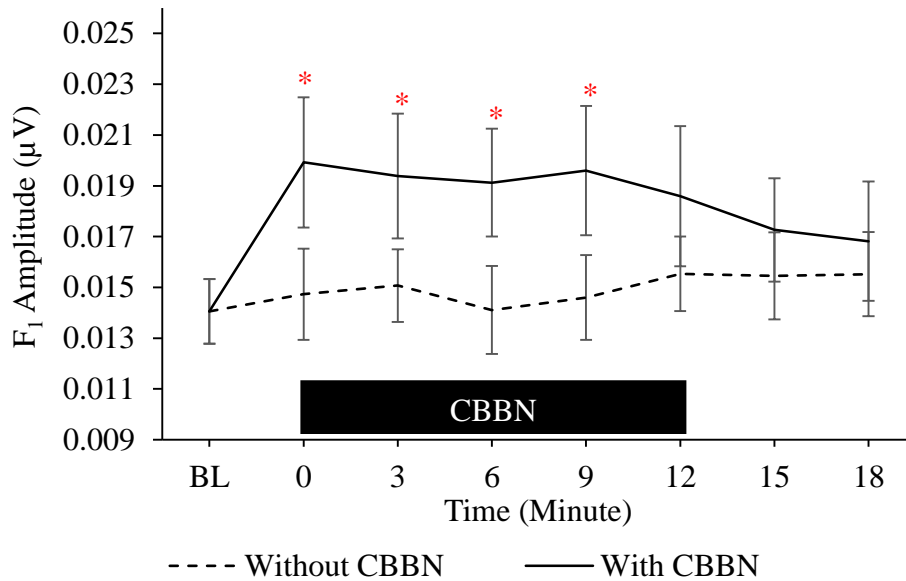


Figure 11. Mean of F_1 amplitude baseline (BL) recording, as well as the amplitude at the seven recording marks without (dashed line) and with CBBN (solid line). Results shown are from a sub-group ($n = 6$) of subjects who received condition (1) first. Asterisks represent the significantly different recordings in comparison to BL. The graph shows that the F_1 increased with the presence of CBBN, and returned to baseline after CBBN was turned off (i.e. minutes 15 and 18), and that F_1 amplitude did not significantly change from BL in the absence of CBBN. The thick line at the bottom of the graph represents the duration of presenting CBBN, in condition (2). (* $p < 0.05$). Error bars represent standard error (± 1 SE).

Table 5. Sub-group post-hoc analysis of F_1 amplitude without and with CBBN. Comparison of F_1 amplitude at each recording mark to baseline (mean = 0.0141 μ V).

Without CBBN			With CBBN		
Recording Mark	Mean (μ V)	<i>p-value</i>	Recording Mark	Mean (μ V)	<i>p-value</i>
0-Minute	0.0147	.56	0-Minute	0.0199	.04*
3-Minute	0.0151	.23	3-Minute	0.0194	.04*
6-Minute	0.0141	.96	6-Minute	0.0191	.04*
9-Minute	0.0146	.64	9-Minute	0.0196	.04*
12-Minute	0.0155	.14	12-Minute	0.0186	.09
15-Minute	0.0154	.13	15-Minute	0.0173	.11
18-Minute	0.0155	.12	18-Minute	0.0168	.20

* $p < 0.05$

Second harmonic for sub-group. Amplitude of $2F_1$ was identified for each recording, similar to what was described above. Repeated measures ANOVA was conducted to compare each recording without CBBN to baseline. Mauchly's test significance could not be determined due to the small sample size, thus, and to be conservative, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.386$). Figure 12 (dashed line) shows that the $2F_1$ amplitude was not significantly different at any recording compared to baseline [$F(2.705, 13.524) = 1.826, p = 0.193, \eta^2 = 0.268$].

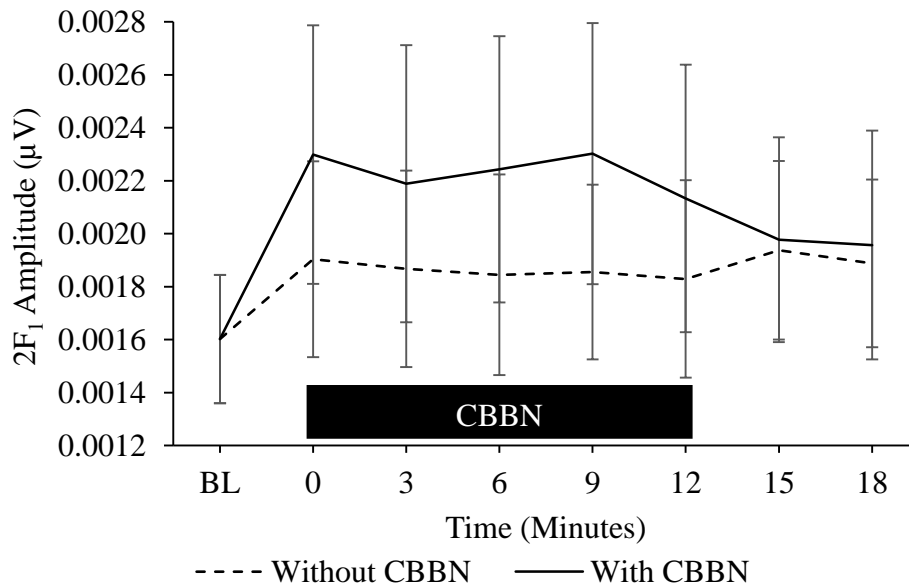


Figure 12. Mean of $2F_1$ amplitude baseline (BL) recording, as well as the amplitude at the seven recording marks without (dashed line) and with CBBN (solid line). Results shown are from a sub-group ($n = 6$) of subjects who received condition (1) first. The graph shows that $2F_1$ amplitude increased with the presence of CBBN, and returned to baseline after CBBN was turned off (i.e. minutes 15 and 18); this increase was marginal, yet not significant. The thick line at the bottom of the graph represents the duration of presenting CBBN, in condition (2). Error bars represent standard error (± 1 SE).

Repeated measures ANOVA was also conducted to compare each recording with CBBN to baseline. Mauchly's test significance could not be determined due to the small sample size,

thus, and to be conservative, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.224$). The results, as illustrated in Figure 12 (solid line), show that the $2F_1$ amplitude was marginal, yet not significantly different at any recording compared to baseline [$F(1.565, 7.823) = 4.262, p = 0.062, \eta^2 = 0.46$]. While the results did not show significant changes, yet the $2F_1$ amplitude is visibly enhanced in the presence of CBBN compared to without CBBN.

Third harmonic for sub-group. Amplitude of $3F_1$ was identified for each recording, similar to what was described above. Repeated measures ANOVA was conducted to compare each recording without CBBN to baseline. Mauchly's test significance could not be determined due to the small sample size, consequently, and to be conservative, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.537$). As shown in Figure 13 (dashed line), $3F_1$ amplitude was not significantly different at any recording compared to baseline [$F(3.762, 18.81) = 1.205, p = 0.34, \eta^2 = 0.194$]. Repeated measures ANOVA was then conducted to compare each recording with CBBN to baseline. Mauchly's test significance could not be determined due to the small sample size, consequently, and to be conservative, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.467$). The results depicted in Figure 13 (solid line) show that $3F_1$ amplitude was significantly different compared to baseline [$F(3.268, 16.342) = 5.71, p < 0.01, \eta^2 = 0.533$]. Post-hoc analysis of recordings with CBBN revealed that $3F_1$ amplitude was significantly different at 0-, 3-, and 6-minute marks, in comparison to baseline, but not at 9-, 12-, 15-, and 18-minute marks, illustrated in table 6. These significant changes occurred during the presentation of CBBN, however, $3F_1$ amplitude returned back to baseline less than nine minutes after the CBBN was turned on. Furthermore, $3F_1$ amplitude slowly approached baseline over time.

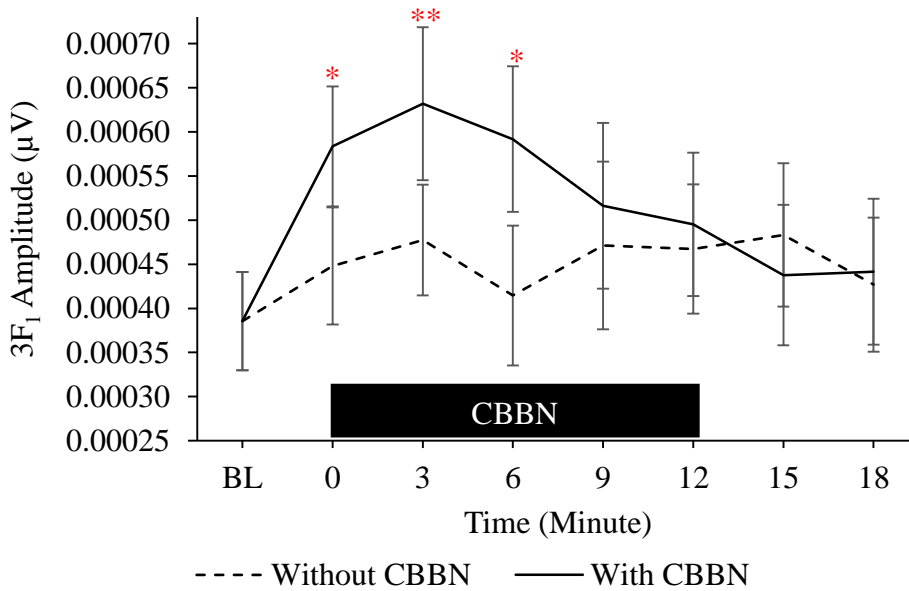


Figure 13. Mean of 3F₁ amplitude of the baseline (BL) recording, as well as the amplitude at the seven recording marks without (dashed line) and with CBBN (solid line). Results shown are from a sub-group ($n = 6$) of subjects who received condition (1) first. Asterisks represent the significantly different recordings in comparison to BL. The graph shows that the 3F₁ increased with the presence of CBBN through the first three (i.e. 0-, 3-, and 6-minute) recordings marks, and returned to baseline over time while CBBN was turned on, and that 3F₁ amplitude did not significantly change from BL in the absence of CBBN. The thick line at the bottom of the graph represents the duration of presenting CBBN, in condition (2). (* $p < 0.05$, ** $p < 0.01$). Error bars represent standard error (± 1 SE).

Table 6. Sub-group post-hoc analysis of 3F₁ amplitude without and with CBBN. Comparison of 3F₁ amplitude at each recording mark to baseline (mean = 0.00039 μV).

Without CBBN			With CBBN		
Recording Mark	Mean (μV)	<i>p</i> -value	Recording Mark	Mean (μV)	<i>p</i> -value
0-Minute	0.00045	.29	0-Minute	0.00058	.013*
3-Minute	0.00048	.01 [§]	3-Minute	0.00063	.009**
6-Minute	0.00041	.64	6-Minute	0.00059	.011*
9-Minute	0.00047	.18	9-Minute	0.00052	.17
12-Minute	0.00047	.09	12-Minute	0.0005	.17
15-Minute	0.00048	.08	15-Minute	0.00044	.24
18-Minute	0.00043	.47	18-Minute	0.00044	.30

* $p < 0.05$, ** $p < 0.01$

[§]Discarded since the main effect was not significant.

CM response phase shift for sub-group. The phase shift of the CM response was identified for each recording, similar to what was described above. Repeated measures ANOVA was used to compare the phase shift for each CM recording without CBBN to the arbitrary baseline of 0 degrees (i.e. no shift). Mauchly's test significance could not be determined due to the small sample size, so, and to be conservative, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.263$). The results, which are illustrated in Figure 14 (dashed line), show that the phase shift was significantly different compared to baseline [$F(1.842, 9.208) = 5.597, p < 0.05, \eta^2 = 0.528$]. Next, repeated measures ANOVA was used to compare the phase shift for each CM recording with CBBN to the baseline of 0 degrees. Mauchly's test significance could not be determined due to the small sample size, so, and to be conservative, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.22$). The results, as shown in Figure 14 (solid line), revealed that the phase shift was significantly different compared to baseline [$F(1.537, 7.686) = 7.977, p < 0.05, \eta^2 = 0.615$]. Post-hoc analysis of recordings without CBBN revealed that phase shift was significantly different at 3-, 6-, 12-, and 18-minute marks, in comparison to baseline, but not at 0-, 9-, and 15-minute marks, illustrated in table 7. Post-hoc analysis of recordings with CBBN revealed that phase shift was significantly different at all recording marks, in comparison to baseline.

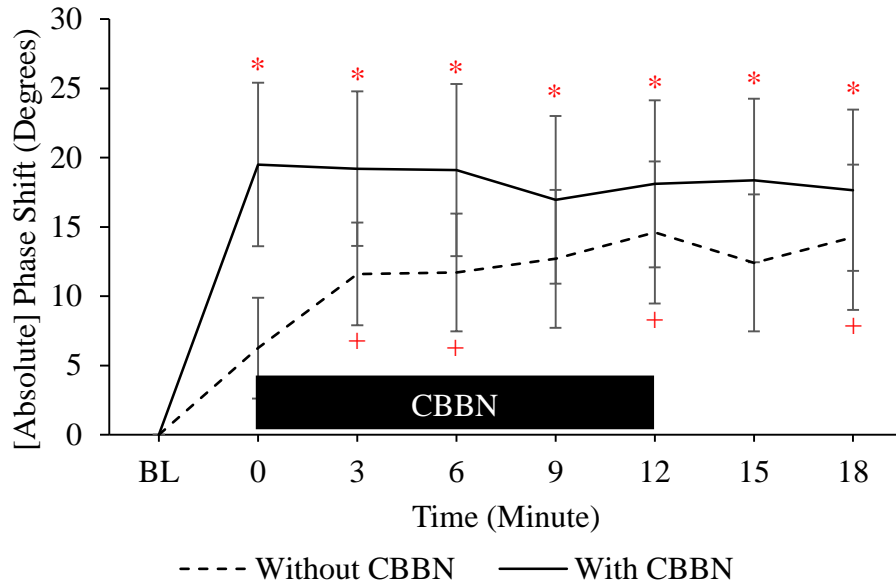


Figure 14. Mean of (absolute) phase shift of the CM response for the seven recording marks without (dashed line) and with CBBN (solid line). Results shown are from a sub-group ($n = 6$) of subjects who received condition (1) first. Pluses represent the significantly different recordings of the without CBBN, and asterisks represent the significantly different recordings of the with CBBN in comparison to BL. The graph shows that there was phase shift observed in all recordings of the with CBBN recordings over time. The thick line at the bottom of the graph represents the duration of presenting CBBN, in condition (2). (* [or +] $p < 0.05$). Error bars represent standard error (± 1 SE).

Table 7. Sub-group post-hoc analysis of phase shift without and with the presence of CBBN. Comparison of phase shift at each recording mark to arbitrary baseline of 0.

Without CBBN			With CBBN		
Recording Mark	Mean (deg)	<i>p</i> -value	Recording Mark	Mean (deg)	<i>p</i> -value
0-Minute	6.25	.15	0-Minute	19.5	.02*
3-Minute	11.6	.03 ⁺	3-Minute	19.2	.02*
6-Minute	11.7	.04 ⁺	6-Minute	19.1	.03*
9-Minute	12.7	.05	9-Minute	16.95	.04*
12-Minute	14.6	.04 ⁺	12-Minute	18.1	.03*
15-Minute	12.4	.05	15-Minute	18.35	.03*
18-Minute	14.25	.04 ⁺	18-Minute	17.65	.03*

* [or +] $p < 0.05$

Chapter IV: Discussion

Several animal and human studies reveal that the MOC efferents play a role in protecting ears from loud sounds, as well as helping with understanding speech in the presence of background noise (Guinan, 2006; Maison, & Liberman, 2000). The current study investigated the effect of activating the MOC fibers on the CM response in humans. The aim of the study is to identify a clinical tool to evaluate the MOC fibers' function, and possibly measure the strength of the MOC reflex. The CM's primary frequency and harmonics were measured with and without activating the MOC fibers for an extended period of time using CBBN.

MOC Fibers Effect on the Primary Frequency (i.e. F1 = 500 Hz)

The findings of the current study demonstrated a significant enhancement of the CM amplitude as a result of activating the MOC fibers using CBBN. The CM response enhancement recorded at 0-minute mark, with CBBN, was $\approx 21\%$ ($0.0215/0.0178 = 120.93\%$) in the overall group, and $\approx 42\%$ ($0.0199/0.0141 = 141.75\%$) in the sub-group of participants receiving condition (1) first. These findings are in agreement with previous findings by Jamos et al. (2012), and it is a well-documented effect in animal studies (Desmedt, 1962; Fex, 1959; Fex, 1967; Gifford & Guinan, 1987; Kemp, & Souter, 1988; Patuzzi, & Rajan, 1990; Sohmer, 1966; Sridhar, Liberman, Brown, & Sewell, 1995; Wiederhold, & Peake, 1966). Fex (1959), Gisselsson and Örebro (1960), and Desmedt (1962) studied the effect of stimulating the OCB on the auditory evoked potentials in cats and guinea pigs. These researchers found similar results of augmented CM response leading them to suggest an increased current shunting through the OHCs. It should be pointed out that acetylcholine (ACh) is the major neurotransmitter released by the MOC neurons, and its role will be discussed in more detail later on. Furthermore, Gisselsson and Örebro (1960) reported that the effect is a result of ACh release by the OCB.

Gifford and Guinan (1987) electrically stimulated the OCB at the floor of the fourth ventricle and showed that CM amplitude increased. Furthermore, Gifford and Guinan recorded CM responses at different click stimulus levels while stimulating the OCB, and observed an amplitude equivalent change of 10 dB at louder levels compared to about a 5 dB change at lower levels. These changes were described based on matching amplitude without stimulating the OCB; in other words, after measuring the CM amplitude while stimulating the OCB, CM response was measured at high levels until similar amplitude was recorded. Therefore, at louder levels, the click stimulus needed to be increased approximately 10 dB to match the amplitude, while at lower levels, only a 5 dB was needed. Patuzzi and Rajan (1990) measured CM in guinea pigs while electrically stimulating the OCB. Similarly, activating the OCB resulted in increased CM amplitude. These researchers provided two hypotheses to explain this phenomenon. Their first hypothesis indicated that the OCB causes reduction in the OHCs length leading to shift in the basilar membrane operating point. However, their data revealed that the basilar membrane is possibly shifted by only 0.7 to 1.3 nm toward the scala vestibuli, which was deemed to be an insignificant change. Their second hypothesis indicated that the activation of the OCB causes a reduction in impedance of the OHCs basolateral wall. After experimentation and theoretical modeling of OHCs electrical circuitry to test this hypothesis, the researchers suggested it to be a possible explanation; especially that the theoretical model used estimated a significant drop of up to 50% in basolateral impedance for high intensity levels. Furthermore, Patuzzi and Rajan suggested that at low intensity levels the OHCs stiffness increases in addition to the drop in basolateral wall impedance.

Lastly, Jamos et al. (2012) conducted a study on human subjects and recorded CMs to 500 and 2000 Hz TB stimuli while presenting CBBN. They reported that the CM's amplitude

increased at 500 Hz, but not at 2000 Hz. They also reported, on average, a 25% increase in amplitude with the presence of CBBN for the 500 Hz TB stimulus. These researchers suggested that, similar to animal data, activation of the MOC neurons creates increased current shunting through the OHCs. This effect is represented by the augmentation of the CM response. The results reported by Jamos et al. are consistent with our current findings.

MOC Fibers Effect over Time. Our results revealed that the increase in the CM amplitude was maintained throughout the 12 minutes of CBBN presentation with no significant changes. After turning off the CBBN, the CM amplitude decayed toward, but never reached, baseline. Our results do not entirely agree with a similar study conducted on animals. Sridhar et al. (1995) attempted to investigate the effect of activating the OCB on CM over time using electric shocks in guinea pigs. They reported that the response was cumulative as a shock was created every 1.5 second, and CM amplitude increased over time. Moreover, these researchers reported that after the OCB stimulation stopped there was a lingering effect that took about 100 seconds to return to baseline. It is worth noting that the lingering effect reported by Sridhar et al. (1995) might be similar to the asymptote effect observed in the current study after turning off the CBBN. Sridhar et al. demonstrated that the lingering effect reported was not a neural effect (i.e. increased firing rate of the MOC fibers), because they severed the OCB directly after stopping the electrical stimulation, and the lingering effect was not altered. However, they suggested that part of this increasing cumulative effect could be associated with the use of electrical shocks, which causes a massive release of ACh by the OCB. It is worth noting that our study used a different method to activate the MOC fibers; we used CBBN (a more natural stimulus) which was presented continuously, rather than electric shocks.

The MOC efferents effect has been studied using DPOAEs in addition to auditory evoked potentials. Kirk and Johnstone (1992) recorded cubic and quadratic difference tones in guinea pigs over an extended period of time. Their results revealed a very small change in the cubic difference tone, while the quadratic difference tone suppression reached up to 12 dB over the time of MOC fibers stimulation (nine minutes). The quadratic difference tone slowly returned back to baseline within five minutes after turning off the CBBN. Furthermore, these researchers were able to block the effect of CBBN using ACh antagonist, Strychnine. Chang and Norton (1997), conducted a very similar study to Kirk and Johnstone, and reported that the quadratic difference tone suppression reached up to 10 dB over a ten minute MOC fibers stimulation, while the cubic difference tone was enhanced by 0.5 dB over time. After turning off the CBBN, the quadratic difference tone reached “asymptote” almost back to baseline within six minutes. These researchers hypothesized that a possible change in the cell length and DC potential that occurs over time causes the asymptote state. Our results show that suppression behavior of the primary frequency does not necessarily agree with the DPOAE data reported in the literature, mostly due to the difference in the generation of each response. However, it is worth noting that both responses reached an asymptote state. Our study also examined the CM distortions (discussed later), which appear to behave in a similar pattern to DPOAEs in response to presenting CBBN.

CM Response Phase Shift. The initial design of the current study did not include the evaluation of phase changes to the primary frequency, as this feature was not known or expected by the researchers. However, during data collection, changes to the CM response phase were observed in comparison to baseline. The phase shift was observed in both the overall group and the subgroup. For the overall group, the phase shift was fairly similar with and without CBBN,

and was between 16 – 19 degrees. The phase shift was maintained over the extended period of time, and did not dissipate after turning off the CBBN. However, for the subgroup of participants receiving the without CBBN recoding first, there was phase shift observed without CBBN, which started at the 3-minute mark and increased slowly over time, and varied between 6 and 14.5 degrees. As with the CBBN recordings, the phase shift was observed from the beginning, and maintained between 17 and 19.5 degrees over the duration of the recording.

After further search in the literature, a study by Mountain, Geisler, and Hubbard (1980) reported a shift of up to 13.5 degrees in CM response phase measured in the 2nd turn of the guinea pig cochlea. Mountain et al. suggested that “a possible explanation of the phase shift is that a change in a circuit-time constant occurred” (p. 239). Kemp and Souter (1988) have reported phase shift of < 20 degrees in CM response between early and late parts of the recording. However, they did not provide explanation to these phase shifts other than stating that the phase shifts they observed were similar to what was observed by Mountain et al. Chertoff (2017 - personal communication) reported observing similar phase shift in some of his animal data. Mills, Norton, and Rubel (1993) measured cubic and quadratic difference tones of CM in scala media while injecting gerbils with I.V. Furosemide. These researchers reported changes to the cubic and quadratic difference tones’ phase, which, they attributed to possible changes to the cochlear amplifier, and that Furosemide interferes with the cochlear amplifier function. A more recent study on humans that used DPOAEs while activating the efferent system also reported phase shift in DPOAEs (Wittekindt, Gaese, & Kössl, 2009). These researchers measured, on average, a 20 degree shift of the $F_2 - F_1$, which was as high as 90 degrees at some recordings. Wittekindt et al. suggested that this change also could be associated with manipulation of the cochlear amplifier, with no additional discussion.

The above findings were not in agreement with a study by Murugasu and Russell (1996). These investigators used a laser diode interferometer to measure the basilar membrane displacement and phase before and during electrically stimulating the OCB in guinea pigs. They did not observe phase changes of basilar membrane displacement as a result of activating the OCB. However, based on the scales used to present the data, it is fairly easy to miss small changes that appear to be on the graph, which are similar to what was seen in the current study (≈ 17 -19.5 degrees) as well as reported by Mountain et al. (1980) and Kemp and Souter (1988). Another possibility to the differences in results could be attributed to the method of measurement used by Margasu and Russell (i.e. laser diode interferometer) in comparison to the phase changes measured using an evoked potential response (i.e. CM).

Another possible explanation to the phase shift is the activation of the stapedial muscle using the CBBN. Sun (2008) studied the effect of presenting CBBN and activating the stapedius muscle on DPOAEs in humans. He presented CBBN below-threshold, at-threshold, and above-threshold of the stapedius muscle contraction. When the CBBN was above the stapedius muscle threshold, the DPOAE amplitude was reduced and phase was changed. The design of the current study was created to avoid such a threat to the validity of the findings. The CBBN we used in the current was presented at 50 dB SPL, and all participants had measurable stapedius muscle threshold above 70 dB HL. Therefore, the impact of stapedius muscle activation in our recordings is unlikely. Additionally, presenting CBBN caused an increase in CM amplitude; if the stapedius muscle was activated, the CM amplitude would have been reduced due to attenuation of the stimulus level reaching the cochlea. We hypothesize that the phase changes observed in this study could be associated with changes of the operating point of the cochlear

amplifier. Further discussion is provided in “The Operating Point (P_0) of the Cochlear Amplifier Modulated by the MOC Fibers” section.

MOC Fibers Modulation of the CM Distortions (i.e. $2F_1 = 1000$ Hz, $3F_1 = 1500$ Hz)

The effect of activating MOC fibers on the nonlinearity of the cochlea has been intensively studied using DPOAEs. However, to our knowledge, the use of CM harmonic distortions to study the MOC efferents has not been used before in humans. It is worth noting that an increase of the CM amplitude (primary frequency) is expected to cause an increase to the CM harmonic distortions, and vice versa. As reported by Dallos (1973), as a function of increasing stimulus level, the primary frequency amplitude of CM response increases. Consequently, the odd and even harmonic distortions’ amplitudes increase as the primary frequency amplitude increases. Therefore, we expected that when the CM response is augmented as a result of MOC fibers activation, the $2F_1$ and $3F_1$ amplitudes are expected to increase. We also hypothesized that any changes in amplitude of the $2F_1$ and $3F_1$ which would occur over time will be of interest to us (see Appendix B for illustration of $2F_1$ and $3F_1$ amplitudes in reference to F_1 amplitude). Especially given that stimulus level used to record CM was not increased.

CM Distortion Modulation of the Overall Group. Our findings revealed a significant enhancement of the CM distortion $2F_1$ amplitude with and without CBBN. This change was similar between conditions (1) and (2), and was maintained throughout the complete 18-minute time-block (keeping in mind that the CBBN was turned off after 12 minutes of continuous presentation). These findings might suggest that the presence of CBBN did not influence the change in $2F_1$ amplitude. As for the CM distortion $3F_1$, the amplitude significantly increased with CBBN, but not in the absence of CBBN. Furthermore, starting at the 6-minute mark and

over time, the amplitude of the $3F_1$ slowly decreased and returned to baseline after the CBBN was turned off. The changes of $3F_1$ amplitude appear to be associated with activating the MOC fibers. Generally, the results of the overall group appear to show a complex relationship between the CM distortions and activation of the MOC neurons. Some of this complexity could be associated with the carry-over effect described in figure 10, especially given that the subgroup data of subjects receiving without CBBN recordings first seem to have not as complex a relationship.

CM Distortion Modulation of the Subgroup. The findings of the current study demonstrated a marginal yet insignificant enhancement of the CM distortion $2F_1$ amplitude with CBBN. However, as shown in Figure 12, the $2F_1$ amplitude was relatively enhanced in the presence of CBBN in comparison to without CBBN. This increased $2F_1$ amplitude was maintained as long as the CBBN was present, then decreased, but never returned all the way to baseline after CBBN was turned off. As for the CM distortion $3F_1$, the amplitude significantly increased in the presence of CBBN, but not in the absence of CBBN. Additionally, the amplitude of the $3F_1$ decreased over time, starting at the 6-minute mark, and approached the baseline by the 9-minute through the 18-minute marks. So, the amplitude returned back near baseline before the CBBN was turned off. Thus, the changes of $3F_1$ amplitude appear to be associated with activation of the MOC fibers. These modulations of the $2F_1$ and $3F_1$ amplitudes seem to be associated with the CBBN. Several published studies used DPOAEs to investigate the effect of activating the MOC efferents on odd and even distortions. Generally, with activation of the MOC efferents the odd order distortion is suppressed, and the even order distortion is enhanced, or vice versa. Our results appear to be in agreement with the pattern of MOC fibers effect reported in the literature.

Several researchers investigated the effect of activating the MOC efferents on the even and odd order distortions DPOAEs (F_2-F_1 and $2F_1-F_2$, respectively) in animals and humans. Brown (1988) conducted a study on guinea pigs and gerbils and reported changes to F_2-F_1 with no change to $2F_1-F_2$. She recorded a reduction in F_2-F_1 amplitude of up to 15 dB over a period of seven minutes, which recovered after turning off primaries. Furthermore, the change in F_2-F_1 amplitude was eliminated when the animals were under deep anesthesia. These results led Brown to suggest that there are different generators for these cochlear distortions, and the observed change of F_2-F_1 amplitude was associated with MOC fibers. Thus, she suggested that activating the MOC fibers affects cochlear mechanics. Another study conducted by Kirk and Johnstone (1993) investigated the effects of activating the MOC fibers on odd and even order distortions in the guinea pig cochlea over a 15-minute long recording. These researchers measured F_2-F_1 and $2F_1-F_2$ while presenting the primary frequencies continuously, with and without CBBN. They observed an overall suppression of F_2-F_1 amplitude of up to 12 dB, which occurred over five minutes of recording, while $2F_1-F_2$ amplitude did not change. Kirk and Johnstone suggested that the operating point was modulated by the MOC efferents causing these changes in the cochlear distortions. It should be noted that the time course of the distortion amplitude change in our results was similar to what has been reported by Kirk and Johnstone. Chang and Norton (1997) also observed similar results, in addition to reporting a slight increase of 0.5 dB of $2F_1-F_2$ amplitude, which lead them to a similar conclusion as Kirk and Johnstone. Furthermore, similar results were reported in humans by Wittekindt et al. (2009), who reported a reduction in F_2-F_1 amplitude up to 4.8 dB, while the $2F_1-F_2$ amplitude did not change. The amount of F_2-F_1 amplitude change increased as a result of increasing CBBN level. These researchers suggested that changes in the operating point or the gain of the cochlear amplifier could explain their

results. A study by Abel et al. (2009) investigated the effect of CBBN on F_2-F_1 and $2F_1-F_2$ amplitude in gerbils. They recorded a similar relationship between distortions, however, it was in the opposite direction compared to results reported by Brown (1988), Kirk and Johnstone (1993), and Chang and Norton (1997). Abel et al. recorded an enhancement in the F_2-F_1 amplitude of about 5.1 dB and a suppression of $2F_1-F_2$ amplitude by about 0.24 dB. Their results appear to show similar pattern to the results of the subgroup of the current study.

The results of the current study and previously conducted studies appear to reflect an interesting relationship between the odd and even order distortions. Additionally, this relationship appears to be influenced by activating the MOC efferents using CBBN. A possible explanation for this relationship may involve mechanical changes happening within the organ of Corti. Thus, it is possible that the activation of the MOC efferents manipulates the operating point of the cochlear amplifier.

The Operating Point (P_0) of the Cochlear Amplifier Modulated by the MOC Fibers

The auditory efferent pathway appears to be of significance to the hearing process, even with the limited knowledge we have about it. MOC neurons and, least understood, LOC neurons are assumed to help with detection of signal in the presence of noise and protection from loud sounds. Furthermore, the strength of the MOC reflex has been connected to the “toughness” of the ear to noise induced hearing loss (NIHL) (Maison & Liberman, 2000). While the physiology for hearing protection is still not entirely clear, the literature appears to agree on the general idea that activating the MOC fibers modulates the cochlear amplifier (Guinan, 2006). The MOC fibers originate in the medial part of the superior olivary complex (SOC) and terminate at the base of the OHCs, i.e. the cochlear amplifier (Guinan, 2006; Maison, Vetter, & Liberman, 2007).

The activation of the MOC efferents appears to have two different effects on the cochlear amplifier: (1) a fast effect that happens within 10 msec, and (2) a slow effect that happens over seconds (Guinan, 2006; Kemp & Souter, 1988; Sridhar et al., 1995). Several explanations have been hypothesized to describe the effect of activating the MOC neurons on the cochlear amplifier. Numerous animal studies and one study on humans suggest that the MOC efferents control the P_0 of the cochlear amplifier, which is demonstrated by modulation of the DPOAEs' amplitudes (Abel et al., 2009; Chang & Norton, 1997; Kirk & Johnstone, 1993; Wittekindt et al., 2009). Other animal studies suggest that the activation of the MOC neurons results in significant reduction of the OHCs impedance, as evident by CM augmentation with no changes to the forward transmission P_0 (Murugasu & Russell, 1996; Patuzzi & Rajan, 1990). The results of the current study on humans appear to support the proposed hypotheses that activating the MOC neurons changes the cochlear amplifier P_0 , and reduces the basolateral impedance of the OHCs. Especially if we use these hypotheses to attempt explaining the fast and slow effects of the MOC fibers.

The Fast Effect of the MOC Fibers. Kemp and Souter (1988) studied the fast effect of activating the MOC efferents on CM in guinea pigs. They reported an increase in the CM amplitude, which began within 8 msec from activating the MOC fibers and reached maximum effect within 10 msec, then declined after the next 200 msec. Our results demonstrated that activation of the MOC fibers results in a fast enhancement of the CM amplitude. This finding was evident by an increase in the primary frequency amplitude at the 0-minute mark, which was recorded as the CBBN was turned on. This enhancement, as discussed earlier, is a result of increased current shunting through the OHCs. The OHCs (i.e. cochlear amplifier) are the major cochlear structure responsible for generating the CM response and innervated by the MOC

neurons (Davis, Deatherage, Eldredge, & Smith, 1958; Guinan, 2006). Therefore, any changes in current flowing through the OHCs, and ultimately mediated by the MOC fibers, could impact the cochlear amplifier. The physiological changes behind this increased CM amplitude have been described extensively in the literature.

Physiologically, when the OHCs are stimulated with an excitatory stimulus that causes the MET channels on the stereocilia to open, a current will be generated. As the stereocilia of the OHCs are surrounded by endolymph, the current generated will be through K^+ , and, slightly, Ca^{+2} (Crawford, Evans, & Fettiplace, 1991). The rush of K^+ to the OHCs depolarizes the cell. Depolarization of the OHC activates the voltage-dependent channels of the cell membrane, including voltage-activated- K^+ and voltage-activated- Ca^{+2} channels (Chambard & Ashmore, 2005). The opening of voltage-activated- K^+ channels remove the K^+ cations from the cell (Chambard & Ashmore, 2005). The opening of voltage-activated- Ca^{+2} channels drives Ca^{+2} into the OHC, and, as a result, deactivates the voltage-activated- K^+ channels (Chambard & Ashmore, 2005). Furthermore, the influx of Ca^{+2} into the cell opens the Ca^{+2} -activated- K^+ channels, which are responsible for hyperpolarizing the cell through increasing K^+ efflux from the OHCs (Dallos et al., 1997; Guinan, 2000; Murugasu & Russell, 1996). This progression of steps happens whenever there is an excitatory signal reaching the organ of Corti. The current flowing through OHCs is responsible for generating the CM response. Now, what happens to this process when the MOC is involved?

The activation of MOC neurons results in release of ACh neurotransmitters, which increases the current flow through the OHCs (Dallos et al., 1997; Elgoyhen et al., 2001; Gisselsson & Orebro, 1960; Murugasu & Russell, 1996; Sridhar et al., 1995). The release of ACh results in an increased influx of Ca^{+2} to the OHCs, which is responsible for activating the Ca^{+2} -

activated K^+ channels (Dallos et al., 1997; Guinan, 2000; Murugasu & Russell, 1996). As a result, the OHCs conductance is increased due to increased efflux of K^+ cations from the OHC, causing hyperpolarization of the OHCs (Dallos et al., 1997; Guinan, 2006; Housley & Ashmore, 1991; Murugasu & Russell, 1996). The decrease in K^+ in the OHCs results in an increase of the apical K^+ influx during depolarization due to the difference in concentration (Dallos et al., 1997; Guinan, 2006; Housley & Ashmore, 1991; Murugasu & Russell, 1996). This effect of ACh leads to increased current shunting, which is quantified by the augmentation of CM response when the MOC fibers are activated. This progression could be a logical explanation for the fast effect of the MOC efferents. It is worth noting that in the current study, the augmentation of the CM response was maintained as long as the CBBN was present. This finding would suggest that the increased current flow through the OHCs was also maintained the whole time CBBN was present. Sridhar et al. (1995) suggested that this fast effect possibly functions as a messenger to trigger the slow effect of the MOC efferents. Moreover, we propose that maintaining the increase in current flow, as is the case in prolonged augmentation of CM, would require a change to the open probability of the MET channels to keep the K^+ flow into the OHC. The question is, how could this be possible?

The Slow Effect of the MOC Fibers. Sridhar et al. (1995) argued that slow, and fast, effects of the MOC efferents result from an intracellular mechanism, especially since both effects were blocked with different ACh antagonists. The reason behind this argument is that when they severed the MOC fibers directly after completing several electrical shocks, the slow effect was still present negating any neural component of this effect. These results led to searching for a possible intercellular change that would result from the release of ACh. As discussed earlier, releasing ACh by the MOC neurons results in Ca^{+2} influx, which increases the intercellular Ca^{+2}

($[Ca^{+2}]_i$) concentration. Sridhar et al., without providing additional explanation, suggested that Ca^{+2} might be a potential second messenger to trigger the slow effect of the MOC efferents. Crawford et al. (1991) demonstrated that increased $[Ca^{+2}]_i$ results in increased probability of the open state of the MET channels, and suggested that $[Ca^{+2}]_i$ is responsible for resetting the P_0 . These results have been demonstrated in other studies as well (Corns, Johnson, Kros, & Marcotti, 2014; Corns & Marcotti, 2016; Wu, Ricci, & Fettiplace, 1999). Moreover, Wu et al. (1999) demonstrated that as $[Ca^{+2}]_i$ is increased, the open probability of the MET channels increased, which created greater shift of the transducer curve without significant change to the slope. However, how do MET channels stay open?

MET channels are located at the shorter stereocilia and are connected by tip-links to the taller stereocilia (Corns et al., 2014; Hudspeth, 1989). MET channels are sensitive to mechanical force, and they open as a result of sheering force applied to the stereocilia (Hudspeth, 1989). It is worth noting that the tip-links insertion structure, at the taller stereocilia, include several molecules. One of these molecules is myosin to facilitate the tension applied on the MET channel to open (Gillespie & Hudspeth, 1993). Gillespie and Hudspeth (1993) attempted to investigate the effect of Ca^{+2} cations on maintaining the opening of the MET channels. They found that Ca^{+2} binds with myosin and causes the open probability of the channels to increase over time (Gillespie, 2004; Gillespie & Hudspeth, 1993). Therefore, it is possible that an increase in the open probability of the MET channels is associated with the increase of $[Ca^{+2}]_i$, as reported by Crawford et al. (1991). In other words, it is possible that Ca^{+2} present inside the cell binds with myosin to increase the open probability of the MET channels. This idea suggests that the P_0 is adjusted by $[Ca^{+2}]_i$ concentration, and that it is shifted toward the scala vestibuli causing an increase in the open probability of the MET channels (Wu et al., 1999). Interestingly enough,

this change happens over time, which makes it a candidate for explaining the slow effect of the MOC efferents. In addition to our study, other studies suggested that the MOC fibers activation results in changes to the P_0 of the cochlear amplifier (Abel et al., 2009; Chang & Norton, 1997; Kirk & Johnstone, 1993; Wittekindt et al., 2009). One method to identify changes to the operating point is through measuring the distortions of the cochlear amplifier. This method could be modeled mathematically and graphically as shown in Figure 15.

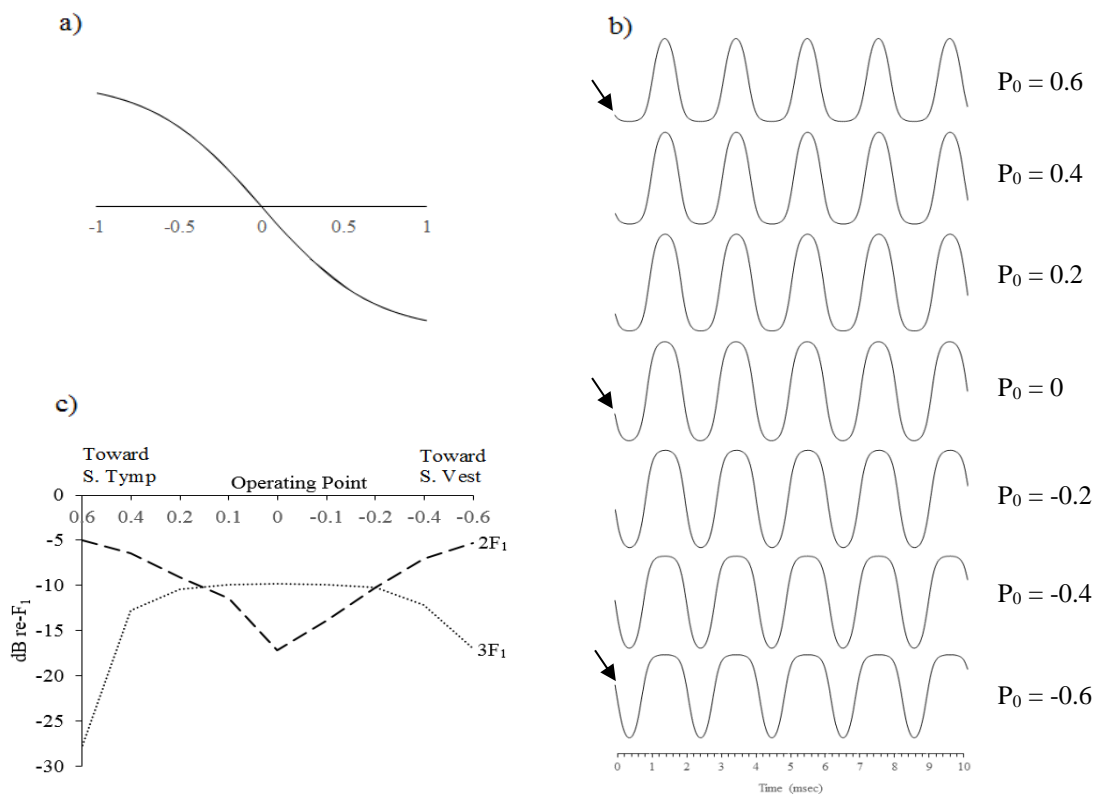


Figure 15. The transducer curve identified from applying the first order Boltzmann function. (a) Transducer curve with 0 operating point (P_0). (b) Output waveforms simulated from the Boltzmann function with different operating points (arrows are pointing to the phase of the waveform). (c) Indicates the relationship between $2F_1$ and $3F_1$ (re. F_1 amplitude) based on the different operating points ($[+P_0]$ – shift toward scala tympani; $[-P_0]$ – shift toward scala vestibuli) (Sirjani et al., 2004, p. 1222).

Figure 15 describes a replication of the mathematical cochlear transducer curve model proposed by Sirjani, Salt, Gill, and Hale (2004). Figure 15(a) is a fitting of the first order

Boltzmann function with P_0 of zero. The Boltzmann function was calculated for different P_0 , and the output waveform was modeled for each of them, as shown in Figure 15(b). Figure 15(c) illustrates the impact of changing P_0 on the relationship between $2F_1$ and $3F_1$, in reference to F_1 amplitude. F_1 , $2F_1$, and $3F_1$ amplitudes were extracted from the output waveforms using FFT analysis. The model described by Sirjani et al. indicates that $2F_1$ and $3F_1$ amplitudes change as a result of changing the transducer P_0 . As P_0 is close to zero, $3F_1$ amplitude is at maxima while $2F_1$ is at minima. Furthermore, as P_0 was shifted away from zero, $2F_1$ gained amplitude while $3F_1$ amplitude was reduced. Additionally, reviewing Figure 15b (arrows) closely, changing the P_0 seem to affect the phase angle of the output waveform, i.e. lead or lag.

The relationship between P_0 and even and odd distortions has been demonstrated in different animal studies using one of two ways: low frequency biasing tone, or injecting gel into the cochlea (Brown, Hartsock, Gill, Fitzgerald, & Salt, 2009; Salt, Brown, Hartsock, & Plontke 2009; Sirjani et al., 2004). The relationship between P_0 and cochlear distortions could be used to explain what has been found in the current study as well as what was reported in the literature. The CM results in the current study revealed that $2F_1$ amplitude was enhanced, while amplitude of $3F_1$ was decreased over time. These results simulate a change to the P_0 that transpires over time, and would lead to possibly explaining the slow effect of the MOC efferents. In addition, the CM response phase shift observed in the current study could possibly be explained by the change in P_0 , as shown in the model in Figure 15b (arrows).

Kirk and Johnstone (1993) suggested that the P_0 changes as a result of activating the MOC fibers due to reduction of the F_2-F_1 amplitude over time, while $2F_1-F_2$ did not change. Chang and Norton (1997) came to a similar conclusion because of a slight increase in $2F_1-F_2$ amplitude and reduction of the F_2-F_1 amplitude. Abel et al. (2009) attempted to bias the P_0 with

and without activating the MOC fibers, and showed that the $2F_1-F_2$ and F_2-F_1 relationship was affected by the biasing tone, then changed again after presenting the CBBN. Furthermore, these researchers reported an enhancement to the even order distortion and a slight suppression to the odd order distortion. These results, in addition to the others in the literature described earlier, support the hypothesis that activating the MOC fibers results in changes to the transducer P_0 . In particular, P_0 is shifted toward the scala vestibuli as a result of increased $[Ca^{+2}]_i$, which could be facilitated by the release of ACh from the MOC neurons.

Another possible source for changes that affect the mechanical responses of the cochlea could originate from the supporting cells of the organ of Corti, specifically Deiters' cells. Deiters' cells appear to receive efferent fibers from the MOC neurons (Nadol Jr. & Burgess, 1994). A recent study by Matsunobu, Chung, and Schacht (2001) reported that Deiters' cells have ACh receptors and can be stimulated via efferent input. Furthermore, the above researchers reported that $[Ca^{+2}]_i$ concentration increased as a result of ACh release over a period of 100 seconds, which in turn, increased the cell stiffness and created a small movement of the phalangeal process head. Matsunobu et al. suggested that these effects could impact the mechanical changes in the organ of Corti. Therefore, a combination of the effect of increased $[Ca^{+2}]_i$ in Deiters' cells and OHCs could be a possible source of the slow effect of the MOC efferents.

Adaptation. At this point, it is worth noting that the current going through MET channels has been shown to experience fast and slow adaptations (Corns et al., 2014; Corns & Marcotti, 2016; Crawford et al., 1991). Fast adaptation occurs within the first 4 msec, while the slow adaptation occurs within 20 msec (Crawford et al., 1991). Several studies demonstrated that fast adaptation occurs as a result of the small Ca^{+2} current going through MET channels (Corns et al.,

2014; Corns & Marcotti, 2016; Crawford et al., 1991). The Ca^{+2} current causes a slight closure of the channel limiting the current going through, and reducing the open probability of the channels (Crawford et al., 1991). As for the slow adaptation, it is possible that structural changes are responsible for reducing the open probability of the channels (Crawford et al., 1991; Gillespie, 2004). Generally, adaptation causes a shift in the transducer curve toward the scala tympani without affecting the slope, making it harder for the channels to open (Cheung & Corey, 2006). However, several studies have demonstrated that increasing the $[\text{Ca}^{+2}]_i$ or removing the Ca^{+2} from the endolymph reduces or abolishes these adaptation effects without affecting the current amplitude (Corns et al., 2014; Corns & Marcotti, 2016; Crawford et al., 1991). Furthermore, as discussed above, the MOC activation increases the influx of Ca^{+2} to the OHC, resulting in increased $[\text{Ca}^{+2}]_i$ and makes adaptation an unlikely source of these changes. As for the current study results, the CM response amplitude only changed with the presence and not in the absence of CBBN. Additionally, the CM amplitude response was augmented as a result of presenting CBBN, which is the opposite of what would be expected with adaptation. Also, the stimulus used in the current study is considered loud (80 dB nHL), which is not expected to create significant adaptation. Wu et al. (1999) showed that adaptation became slower, and not as pronounced, for stronger stimuli in comparison to smaller stimuli (smaller bundle displacement).

The MOC Fibers and Protection from Noise Exposure

Excessive noise exposure is becoming an increasing problem, and is known to cause damage to the auditory system, causing temporary or permanent hearing loss. A study conducted by the CDC estimated that about 40 million people above the age of 24 have been exposed to excessive levels of noise, and have unilateral or bilateral NIHL (NICDC, 2017). Furthermore, prevalence of NIHL in US youth is also on the rise, with about 16% of youth between 12-15

years old and 17.7% of youth between 16-19 years old estimated to have hearing loss (Henderson, Testa, & Hartnick, 2011). NIHL could be from the result of occupational noise exposure, or recreational noise exposure such as firearm usage and/or listening to loud music with limited use of hearing protection (Henderson et al., 2011). Exposure to damaging amounts of noise is incredibly common, and it is very important to identify patients who are at a higher risk for developing hearing loss as a result of this exposure.

The auditory system appears to employ some protective process against noise exposure, which appears to be different from one individual to another. One potential process is the MOC reflex, which is responsible for modulating the cochlear amplifier (Guinan, 2006). Several animal studies have demonstrated the importance of the MOC reflex to protect from noise exposure (Maison & Liberman, 2000; Maison, Luebke, Liberman, & Zuo, 2002; Rajan, 1988a; Rajan, 1988b). Rajan (1988a, b) measured the effect of MOC fibers activation on temporary threshold shift (TTS) in guinea pigs. The results showed that activating the MOC fibers electrically resulted in reduced TTS compared to control, and TTS was recorded again after damaging the MOC fibers. Those results led the researcher to conclude that the MOC reflex has a protective function from exposure to loud sounds. This effect appears to be related to the strength of the stimulus used to activate the MOC fibers. That is, the stronger the MOC fibers activator, the smaller the TTS (Rajan, 1988a). Also, the delay between noise exposure and activating the MOC fibers seems to impact the MOC protective function; simultaneous activation of MOC with noise exposure caused the smallest TTS (Rajan, 1988a). Masion and Liberman (2000) evaluated the strength of the MOC reflex based on the amount of suppression of DPOAEs in guinea pigs, and divided them into three groups: weak ($\approx 3\text{-}5$ dB), intermediate ($\approx 8\text{-}10$ dB), and strong ($\approx 14\text{-}15$ dB). All three groups were exposed to sounds loud enough to cause a

permanent threshold shift (PTT). Their results revealed that the animals with the strong MOC reflex demonstrated the lowest amount of PTT (10-15 dB), while the animals with a weak MOC reflex showed a significant PTT (30-50 dB). These results led Masion and Liberman to believe that the strength of the MOC reflex could be used to describe ears as “tough vs. tender”. However, they did not explain what might cause these differences in MOC reflex strength.

The current study, while not attempting to measure any noise exposure effects, revealed that CM responses could be used as a tool for measuring the strength of the MOC reflex. As mentioned above, the activation of the MOC fibers using CBBN resulted in a significant increase in the CM amplitude. Furthermore, and as shown in Figure 16, individual data showed the amplitude changes to be negligible in some subjects while large in others. These individual differences appear to be in agreement with previously published data on the different strength of MOC reflex (Masion & Liberman, 2000). (Note. See appendix C for MOC reflex data of all participants in the study).

Generally, these results suggest that the MOC efferents control the cochlear amplifier as part of a feedback loop, which has different impact based on individual differences (Masion & Liberman, 2000). In the presence of loud sound, a large influx of K^+ will be allowed into the OHC. While K^+ is very important to the hearing process, the accumulation of this ion in the OHCs with reduced efflux could have negative impacts on the cellular structure. Kharkovets et al. (2006) reported that the loss of major OHC K^+ efflux would result in progressive degeneration of the OHCs. As discussed earlier, the activation of the MOC neurons helps removing K^+ from the cell through increasing conductance of the basolateral wall. Therefore, we propose that a strong MOC reflex would lead to greater current shunting through the OHCs, represented in larger CM enhancement, and as a result, a “tougher” ear. However, a weak MOC

reflex might not create a significant effect to the current shunting through the OHCs, represented in minimal to no CM enhancement, resulting in a “tender” ear.

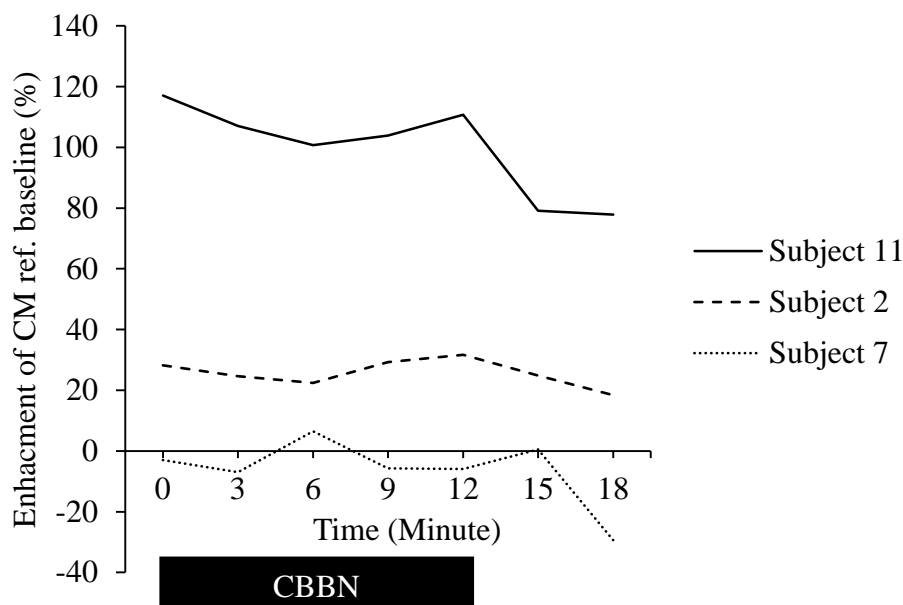


Figure 16. Three examples of subjects with different MOC reflex strengths. The y-axis is indicating the enhancement of CM response in reference to the subject baseline for the recording with CBBN [$100 \% * (CM \text{ amplitude} - \text{Baseline}) / \text{Baseline}$], and x-axis is indicating time. Subject 11 (solid line) is showing a large effect of over doubling in amplitude. Subject 2 (dashed line) is showing a moderate enhancement of around 30%. Subject 7 (dotted line) does not indicate any enhancement effect. The thick line at the bottom represents the duration of presenting CBBN.

The differences between “tough” and “tender” ears could originate from genetic factors that make some patients more vulnerable to noise exposure than others (Nouvian et al., 2003; Sliwiska-Kowalska & Pawelczyk, 2013; Van Laer et al., 2006). A study conducted by Maison et al. (2002) demonstrated that an increase in expression of $\alpha 9$ nicotinic cholinergic receptor in a mouse model revealed stronger MOC reflex and made animals more resistant to NIHL. Thus, we propose that measuring CM enhancement could be used as a tool to identify patients who are at risk for developing NIHL. We further propose that recording the CM response enhancement could be completed over time to monitor for changes in the OHCs function as a result of noise

exposure. The question to be answered here is: What is the feedback loop that is responsible for controlling the cochlear amplifier?

Feedback Loop of the Cochlear Amplifier. The cochlear amplifier is responsible for amplifying soft sounds, and possibly attenuating loud sounds. Figure 17 shows the feedback circuit of the cochlear amplifier represented by the efferent pathway. This circuit consists of an ascending (afferent) pathway represented by the Type II fibers, and a descending (efferent) pathway represented by the MOC fibers.

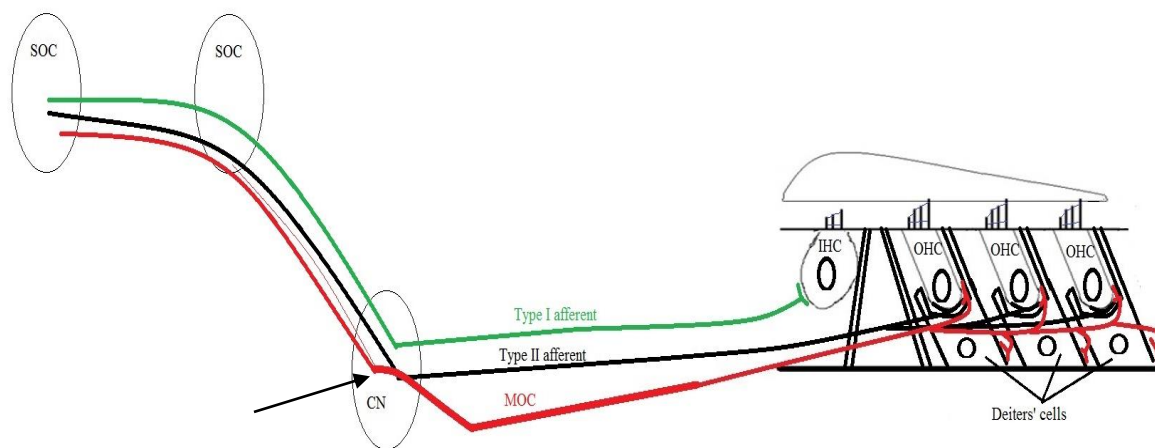


Figure 17. The feedback circuit of the cochlear amplifier. Arrow pointing to the overlap between Type II afferents and MOC efferents. SOC—superior olivary complex, CN—cochlear nucleus, IHC—inner hair cell, OHC—outer hair cell, MOC—medial Olivocochlear.

Recently, Type II afferent fibers have been shown to drive the MOC reflex in mice (Froud et al., 2015). This study utilized a mouse model that lacked the gene ($-/- Prph$) responsible for developing Type II afferents, and compared the results to wild-type littermates. Results indicated that the hearing threshold between both types of mice were within normal limits with no differences between null and wild-type mice. However, the MOC reflex, ipsilateral and contralateral, was absent in the null ($-/- Prph$) mice, while preserved in the wild-type ($+/+ Prph$) littermates. Moreover, Type II afferent fibers have been shown to receive

glutamate neurotransmitters released from the OHCs (Zhang & Coate, 2017; Weisz, Lehar, Hiel, Glowatzki, & Fuchs, 2012). Additionally, basal Type II afferents, in comparison to Type I afferents (resting potential of -43 mV), are more hyperpolarized with a resting potential of -54 mV and have a longer latency to fire (Reid, Flores-Otero, & Davis, 2004). Reid et al. stated that these firing characteristics, in addition to the innervation pattern to OHCs (one-to-many), help with detection of soft level sounds. Furthermore, Zhang and Coate (2017) reported that painful sounds have been shown to activate the Type II fibers, which is completed through more of an ATP than glutamate release. However, Type II fibers, along with Type I fibers, carry on the signal to higher levels of the auditory system where they could participate in activating the MOC fibers at the SOC level. Interestingly enough, Brown and Ledwith (1990) reported that Type II afferents overlap with the MOC fibers in the border area between the ventral and dorsal cochlear nuclei, yet it is unclear if they affect each other's function at that level.

With the presence of a loud sound, a signal will be sent down through the MOC neurons to the OHCs and the Deiters' cells resulting in release of ACh (Matsunobu et al., 2001; Nadol Jr. & Burgess, 1994). As reported in the literature, the strength of the electric shock applied to the MOC fibers controls the strength of the reflex (Desmedt, 1962; Rajan, 1988a). The strength of the MOC reflex could be due to different levels of ACh release; stronger stimuli result in greater release of ACh. As described above, the release of ACh results in increased current flowing through the OHCs due to the increased conductance of the basolateral wall of the OHCs. Pattuzi and Rajan (1990) described the OHCs as a series of resistors; the apical transduction as a variable resistor and the basolateral wall as a preset resistor. Additionally, these researchers suggested that activation of the MOC fibers results in dropping the preset resistor (impedance of the basolateral wall), leading to a reduction of the driving current of the OHCs. To add to this

rationale, we propose that the variable resistor (apical transduction impedance) also drops as a result of changing the P_0 over time. Significantly dropping the impedance of the OHCs circuit could possibly create a short circuit that would significantly reduce or eliminate the amplifier function in the presence of a loud sound.

Chapter V: Study Limitations

The current study is designed to investigate the effect of activating the MOC fibers over a period of time. CM was measured in four time-blocks of 18-minute long, two without [condition (1)] and two with CBBN [condition (2)]. The order of recording was randomly selected for each subject from the following combinations: YYNN, YNYN, YNNY, NYYN, NYNY, and NNNY. To evaluate the different aspects of the MOC reflex over time, we evaluated the decay of the MOC reflex after CBBN was turned off. CBBN was turned off after 12 minutes of continuous presentation in condition (2), which allowed for two recordings at the 15- and 18-minute marks to measure the decay of the response. In addition, subjects were given a five-minute break between each two time-blocks to allow additional time for the MOC reflex to dissipate. The break between time-blocks and the last six minutes in condition (2) time-blocks do not have CBBN, and would add up to 11 minutes of time with no activation of the MOC fibers. This period of time was considered adequate, especially given that other studies on DPOAEs have demonstrated that the response returns to baseline within about five to six minutes (Chang & Norton, 1997; Kirk & Johnstone, 1993). During data analysis, five out of ten subjects who received condition (2) first, showed a carry-over effect into the condition (1) recordings. As shown earlier in Figure 10, the carry-over effect was present as a similar increase in amplitude that was recorded in both conditions, (1) and (2). This finding was unexpected, yet it could be related to the difference in the way CM and DPOAE are generated. While this condition caused some limitation to the study, the analysis was completed on a subgroup of subjects ($n = 6$) who received condition (1) first. The subgroup was still very informative, and provided us with valuable information.

It was difficult to compare our findings with other studies investigating MOC reflex and CM since the majority of them have been performed using animals. Activating the MOC fibers in animals is different compared to humans. In animals, the MOC neurons could be activated by direct electrical stimulation at the floor of the fourth ventricle, which is very invasive. On the other hand, in humans, the MOC fibers can be activated using an acoustic, not electric, stimulus. However, we believe that using an acoustical stimulus to activate the MOC fibers provides a better simulation of daily life. Lastly, there was one study available on the effect of MOC reflex on odd and even distortions in humans conducted by Wittekindt et al. (2009), which was completed using DPOAEs, and was in fair agreement with our results.

Chapter VI: Conclusions

The MOC efferents are thought to protect hearing from loud sounds. The current study attempted to provide additional information about the physiological changes resulting from activating the MOC fibers, and to identify a clinical tool to evaluate MOC reflex in humans. The effect of activating the MOC fibers on CM response distortions were measured over time, and revealed that CM distortions are modulated over time. Changes of the CM response distortions could be interpreted as a result of altering the operating point of the cochlear amplifier modulated by the MOC efferents.

Additionally, our results revealed that CM amplitude is enhanced as a result of activating the MOC fibers using a CBBN. Our results suggest that the recording of CM response with and without CBBN could be used as a tool to evaluate the strength of the MOC reflex in humans. In the meantime, recording the CM response can be somewhat challenging due to their vulnerability to artifacts and limited clinical use. However, clinicians can employ several techniques to ensure recording the cochlear response without artifact, such as: insulating the insert earphones, recording a control run with pinched tube, increasing the insert earphone tube length, and keeping the electrode lead and the insert earphone wire far apart. This clinical application of CM response can enhance the clinical value of this cochlear response. Regarding the method of recording, this clinical application will be better recorded using TM electrode to obtain a robust amplitude, which might require additional training to clinicians for placing the electrode. However, this technique is used fairly often in clinical setting to record the Electrocochleography response. While our study supports the use of CM to evaluate MOC reflex, additional research is needed to identify optimal parameters for this particular clinical approach.

Chapter VII: Literature Review

The auditory efferent system is a neural pathway that extends from the central auditory nervous system, the superior olivary complex, to the peripheral auditory system, the cochlea. This pathway consists of the MOC fibers synapsing with the OHCs, and the LOC fibers synapsing with Type I afferents of the inner hair cells (Guinan, 2006). The efferent system is thought to help with understanding speech in noise and protecting against loud noises (Guinan, 2006). The physiology of the MOC efferents is not entirely clear, however, several researchers have suggested that it is responsible for modulating the function of the cochlear amplifier (Guinan, 2006; Wittekindt et al., 2009).

The cochlear amplifier, as represented by the OHCs, is a nonlinear system. The nonlinearity of the cochlear amplifier is responsible for generating cochlear distortions, which were not part of the input signal (Brown et al., 2009; Salt, Brown, Hartsock, & Plontke, 2009; Wittekindt et al., 2009). The cochlear distortions, even order (i.e. $F_2 - F_1$ or $2F_1$) and odd order (i.e. $2F_1 - F_2$ or $3F_1$), have specific relationships with the operating point of the cochlear amplifier transducer curve. The operating point is defined as the point where the stimulus is crossing the resting position “0” pressure, or when the organ of Corti is at the resting position (Salt et al., 2009). Therefore, when the operating point is at zero, the transducer curve will be symmetrical, which results in a large odd order distortion amplitude while the even order distortion is at its minimum (Brown et al., 2009; Salt et al., 2009; Sirjani, Salt, Gill, & Hale, 2004). Furthermore, when the operating point is moved away from the resting position, toward scala vestibuli or scala tympani, the transducer curve will no longer be symmetrical. This change impacts the cochlear distortions, the even order distortion will increase in amplitude while the odd order will decrease in amplitude (Brown et al., 2009; Salt et al., 2009). Therefore, the position of the organ of Corti

operating point is important for the function of the cochlear amplifier, and this process is a possible way for the MOC efferents to modulate the cochlear amplifier by adjusting the operating point.

Pattuzi and Rajan (1990) investigated the effect of stimulating the crossed OCB on the position of the organ of Corti. These researchers recorded the CM response to a 200 Hz signal in pigmented guinea pigs and constructed transducer curves based on the CM responses. Also, they compared their data to a fitted model, which they created using the first order Boltzmann function. Pattuzi and Rajan showed that the CM amplitude increased by up to 60% as a result of electrical stimulation of the OCB; however, the CM response was saturated at louder levels (110 – 115 dB SPL) with an increase of up to 20%. Using the CM response, these researchers constructed transducer curve, which had Lissajous shape, by plotting the input pressure with the associated voltage change of the OHCs. They constructed transducer curves with and without stimulating the OCB electrically, and concluded that the Lissajous characteristics, shape, and inflection points did not change when stimulating the OCB. Based on this observation, Pattuzi and Rajan claimed that the OCB stimulation did not change the operating point of the organ of Corti.

Kirk and Johnstone (1993) disagreed with the results reported by Pattuzi and Rajan (1990). Kirk and Johnstone investigated changes in F_2-F_1 and $2F_1-F_2$ in guinea pigs while activating the MOC fibers over an extended period of time. They presented contralateral noise for a duration of 5 to 10 minutes, and measured F_2-F_1 and $2F_1-F_2$ distortion amplitudes at 1-minute intervals. Their results showed that F_2-F_1 amplitude decreased over time by about 12 dB, then recovered back to the original level after five minutes of stopping the noise. As for $2F_1-F_2$, amplitude decreased by about 2 dB over time, and did not recover after stopping the noise. Kirk

and Johnstone then injected the animals with a cholinergic blocker, Strychnine, to block the MOC neurons function, which caused the F_2-F_1 changes to disappear. These results confirm that the observed changes in F_2-F_1 were due to activating the MOC fibers. The researchers suggested that the reported effect could be a result of changes in the angle of stereocilia and possibly the change in the operating point. Kirk and Johnstone's results were confirmed by Chang and Norton (1997) who ran a similar study on guinea pigs.

Abel et al. (2009) investigated the MOC fibers' influence on the operating point in Mongolian gerbils using DPOAEs. These researchers used the relationship between the operating point and even and odd order distortions to evaluate the MOC fibers function. They used the second order Boltzmann function to construct a hypothetical model of the transducer function. Abel et al. measured the change in distortions' amplitudes in three conditions: stimulating the MOC efferents, biasing the organ of Corti using a 5 Hz tone, and simultaneously biasing the organ of Corti and stimulating MOC efferents. Results showed that with presentation of white noise to the contralateral ear, F_2-F_1 amplitude showed a significant increase, while $2F_1-F_2$ amplitude was not affected. The amount of change in F_2-F_1 amplitude increased with increased levels of white noise in the contralateral ear; the change in amplitude averaged at 5.1 dB, with change recorded as high as 10.4 dB. The low frequency biasing experiment revealed a double modulation of the distortions' amplitude due to modulating the operating point of the organ of Corti. When the white noise was introduced to the contralateral ear, the modulation changed from a double dip to a single dip in the F_2-F_1 amplitude. Abel et al. argue that changes in the modulation pattern are due to the ability of the MOC fibers to reset the operating point of organ of Corti.

In humans, Wittekindt et al. (2009) conducted a study to evaluate the effect of activating the MOC efferents on the even and odd order distortions using the DPOAE. They recruited 23 young, normal hearing adults, and attempted to record F_2-F_1 and $2F_1-F_2$ from all subjects. However, because of the difficulty recording F_2-F_1 in the lower frequency range due to background noise, only seven subjects were able to complete the study. For the DPOAE recording, researchers used $F_2 = 5000$ Hz while varying the F_1 frequency to allow for a robust F_2-F_1 response; F_2-F_1 frequency ranged from 833 to 1429 Hz. The recording was completed over three, second-long intervals: first and third intervals were recorded without stimulating the MOC fibers, while the second interval was recorded while presenting contralateral broadband noise (CBBN). Wittekindt et al. recorded F_2-F_1 while presenting CBBN for 30 seconds in only one subject. Results revealed about a 4.5 dB reduction of F_2-F_1 on average with the presence of CBBN, while $2F_1-F_2$ did not show any changes. Also, these researchers reported a change in the distortion phase as a result of presenting CBBN. When they attempted to measure the CBBN effect over a longer duration, the F_2-F_1 amplitude was suppressed within about 0.6 seconds, then slowly increased over the following 10-15 seconds. The results of this study can be considered exploratory due to the number of subjects and the difficulty in recording the F_2-F_1 response; especially that it was the only study that looked at the effect of MOC neurons on the cochlear even order distortion in humans.

In 2012, Jamos et al. conducted a study to investigate the effect of activating the MOC neurons on the CM response in humans. The researchers investigated the effect of ipsilateral versus contralateral activation of the MOC fibers using different levels of broadband noise and different toneburst frequencies, low vs high frequencies. The CM response amplitude and the power spectrum of the response were analyzed. Jamos et al. reported that the amplitude of the

CM response for the low frequency stimulus (i.e. 500 Hz) was enhanced in about 83% of participants in the condition of contralateral stimulation at all broadband noise levels, with 50 dB SPL causing the largest amount of enhancement. The results also revealed a significantly larger effect at the lower frequency compared to the higher frequency. These findings suggested that CM could be an appropriate tool to measure low frequency distortions, which is one thing that might be difficult to investigate and record if the OAEs were used. Jamos et al. focused on the energy of the power spectrum at the frequency region tested (i.e. 500 vs. 2000 Hz). The results showed increased energy at the tested frequency region in all conditions, with greater enhancement at the lower frequency and in the contralateral stimulation conditions. At the time, researchers did not investigate the changes to the cochlear distortions.

In summary, the MOC fibers have been shown to modulate the cochlear responses; however, the way they function is still not entirely understood. The MOC neurons function has been investigated extensively in animals using evoked potentials and OAEs. In humans, several studies have investigated the MOC neurons function using OAEs, mainly the DPOAE at $2F_1-F_2$ (Abdala, Mishra, & Williams, 2009; Moulin, Collet, & Duclaux, 1993). However, OAEs recording has some limitations, including: limited information at the lower frequencies (such as F_2-F_1) due to background noise and the apparently small effect of efferent suppression (Moulin et al., 1993; Wittekindt et al., 2009). In recent years, the use of CM has gained more attention in studying the efferent system in humans, although it requires longer testing times and it is slightly more invasive compared to OAEs (Jamos et al., 2012). Our current study was a continuation of the work by Jamos et al.; however, this study focused on the cochlear distortions recorded by the CM response.

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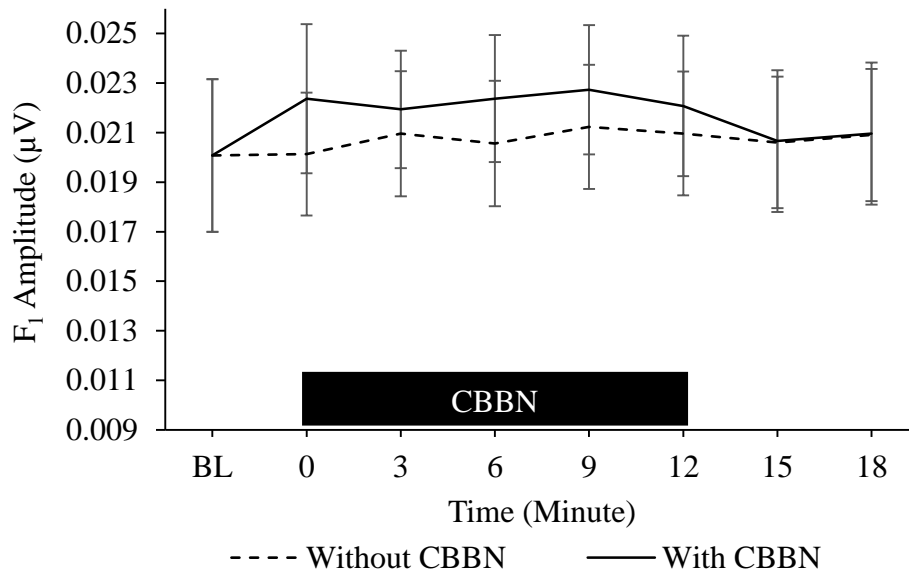
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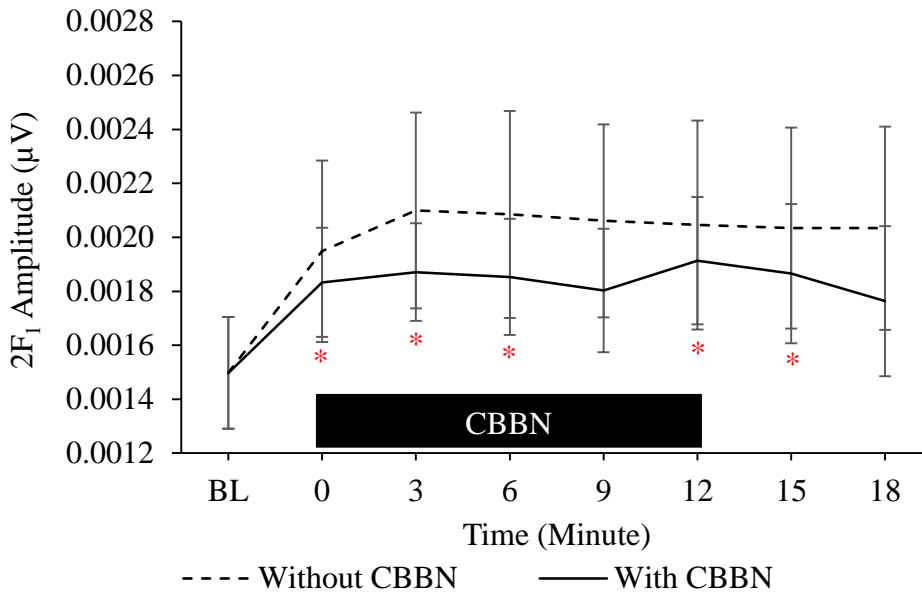
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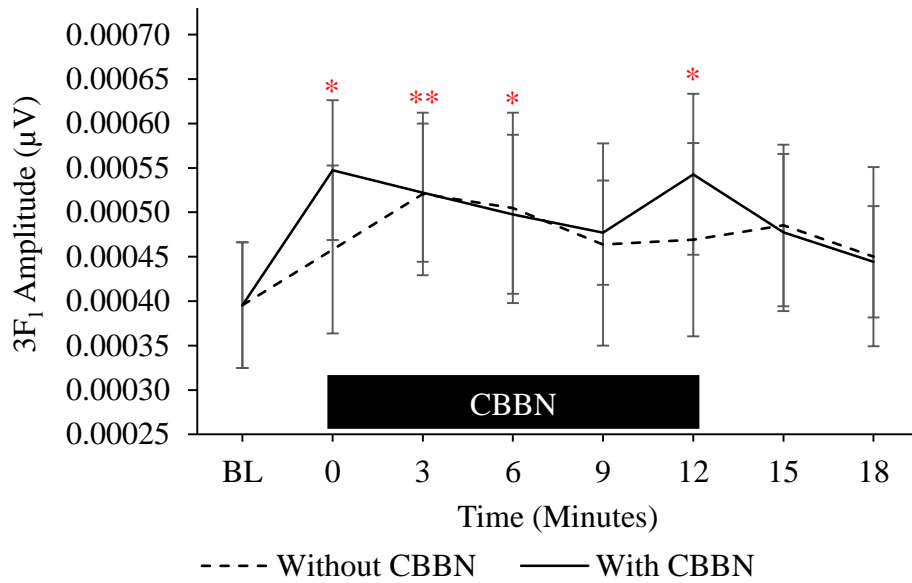
Appendix A: Sub-group Subjects Who Received Condition (2) First



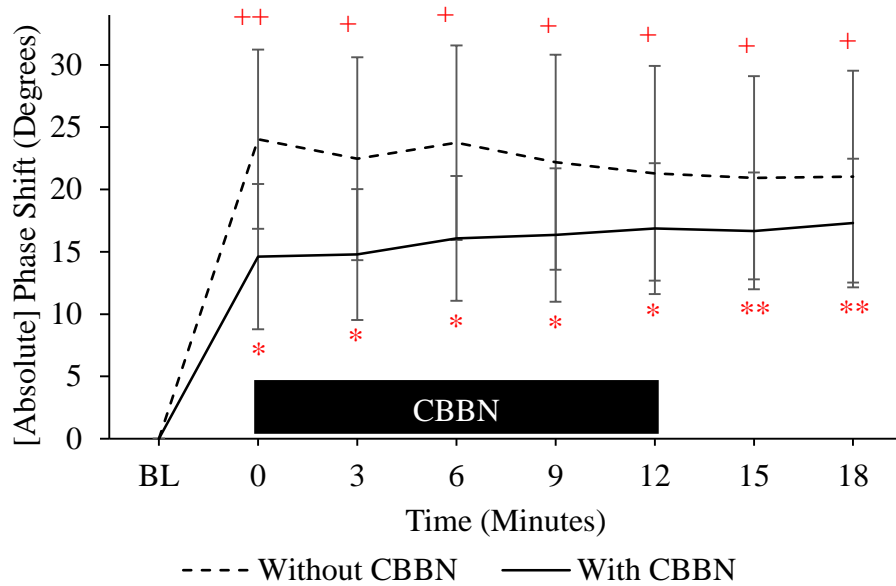
Supplemental Figure 1. Mean of F_1 amplitude baseline (BL) recording, as well as the amplitude at the seven recording marks without (dashed line) and with CBBN (solid line). Results shown are from a sub-group ($n = 10$) of subjects who received condition (2) first. The graph shows that the F_1 increased with the presence of CBBN, and returned to baseline after CBBN was turned off (i.e. minutes 15 and 18). The thick line at the bottom of the graph represents the duration of presenting CBBN, in condition (2). Error bars represent standard error (± 1 SE).



Supplemental Figure 2. Mean of 2F₁ amplitude baseline (BL) recording, as well as the amplitude at the seven recording marks without (dashed line) and with CBBN (solid line). Results shown are from a sub-group ($n = 10$) of subjects who received condition (2) first. The graph shows that the 2F₁ amplitude increased in both conditions, (1) and (2). The thick line at the bottom of the graph represents the duration of presenting CBBN, in condition (2). (* $p < 0.05$). Error bars represent standard error (± 1 SE).

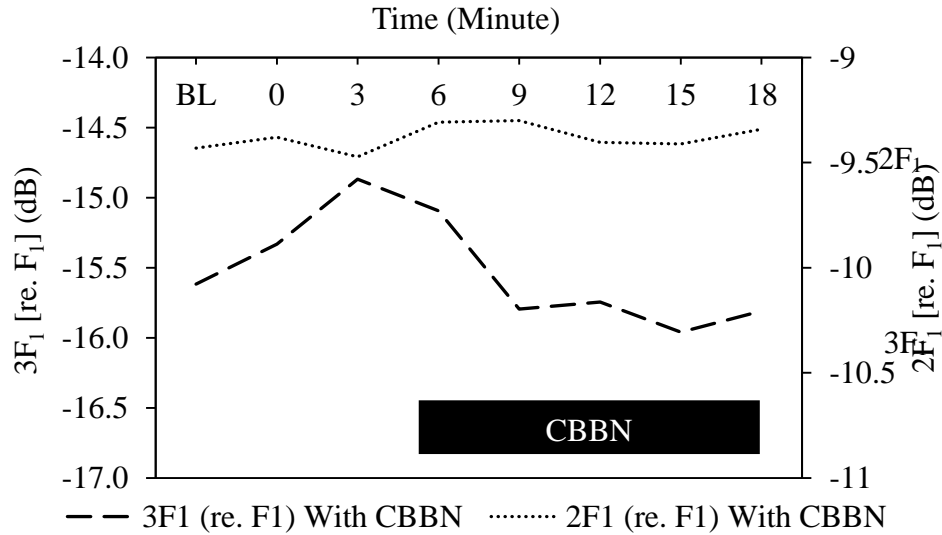


Supplemental Figure 3. Mean of 3F₁ amplitude baseline (BL) recording, as well as the amplitude at the seven recording marks without (dashed line) and with CBBN (solid line). Results shown are from a sub-group ($n = 10$) of subjects who received condition (2) first. The graph shows that the 3F₁ amplitude fluctuated with and without the presence of CBBN. The thick line at the bottom of the graph represents the duration of presenting CBBN, in condition (2). (* $p < 0.05$; ** $p < 0.01$). Error bars represent standard error (± 1 SE).



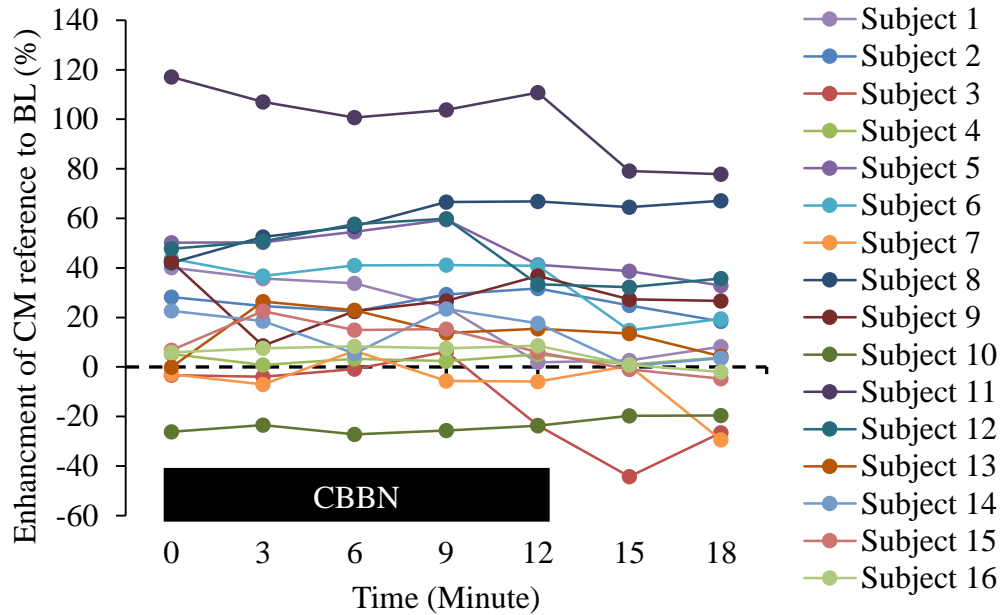
Supplemental Figure 4. Mean of (absolute) phase shift of the CM response for the seven recording marks without (dashed line) and with CBBN (solid line). Results shown are from a sub-group ($n = 10$) of subjects who received condition (2) first. The graph shows that there was a phase shift observed in all recordings with and without CBBN over time. Pluses represent the significantly different measurements of the without CBBN recordings, and asterisks represent the significantly different measurements of the with CBBN recordings in comparison to BL. The thick line at the bottom of the graph represents the duration of presenting CBBN, in condition (2). (* [or +] $p < 0.05$; ** [or ++] $p < 0.05$). Error bars represent standard error (± 1 SE).

Appendix B: Amplitude Ratio of Sub-group Subjects Who Received Condition (1) First



Supplemental Figure 5. Mean of 2F₁ amplitude (dotted line) and 3F₁ amplitude (dashed line) in reference to F₁ amplitude [re. F₁] over time. The graph illustrates a logarithmic ratio calculated using $(10 \times \log_{10} [\text{distortion}/F_1])$. This data is for the sub-group who received condition (1) first ($n = 6$). The graph indicates minor fluctuations of the 2F₁ amplitude, while 3F₁ amplitude was decreased over time. The thick line at the bottom of the graph represents the duration of presenting CBBN.

Appendix C: CM Amplitude Enhancement (%) for All Subjects



Supplemental Figure 6. The MOC reflex strength plotted for all participants in the study. The y-axis is indicating the enhancement of CM response in reference to the subject baseline for the recording with CBBN [$100 \% * ((\text{CM amplitude} - \text{Baseline}) / \text{Baseline})$], and x-axis is indicating time. The greater the enhancement, the stronger the MOC reflex. The thick line at the bottom represents the duration of presenting CBBN.